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EMBRYOLOGY OF CERTAIN RANALES

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(WITH EIGHTEEN FIGURES)

Studies in the embryology of various members of the Ranales have been made from time to time, but no one seems to have called attention to the similarities and dissimilarities that occur in the group as a whole. Of the five families that have been investigated, it is of interest to note that the embryology of the Magnoliaceae, Ranunculaceae, and Berberidaceae conforms to one general plan which is quite different in certain respects from another rather uniform plan found in the Nymphaeaceae and Ceratophyllaceae. The present investigation of the embryology of *Magnolia grandiflora* L. and *Cimicifuga racemosa* (L.) Nutt. furnishes additional evidence as to the uniformity of embryo development in the first three families.

Investigation

MAGNOLIA GRANDIFLORA

Magnolia grandiflora is native to the coastal plain of the southeastern United States, but is successful in cultivation farther inland. Material examined for this paper was collected at Greenville, South Carolina, at intervals from the first of May until the latter part of September, in 1933. In the northwestern part of South Carolina, where Greenville is situated, flowering begins about the first of May and newly opened flowers may be found as late as the end of July, so that ovules in various stages of development may be collected simultaneously.

The many unicarpellate pistils of a flower, arranged spirally upon a cone-shaped receptacle, each contain two anatropous ovules, one or both of which may develop into a seed. Each pistil develops into a small follicle at maturity, and the aggregation of follicles on the receptacle forms a conelike structure about 3 to 5 inches in length and about 2 to 2.5 inches in diameter. When mature, the follicles dehisce and allow the seeds to escape, although for a time they remain attached to the follicle by means of a slender strand of protoxylem, the remains of the funiculus. This eventually breaks and the seed falls to the ground; or it sometimes happens that the entire cone with the attached seeds is shed.

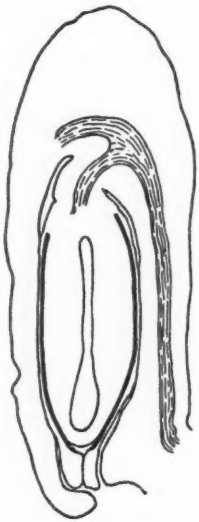


FIG. 1.—Median longisection of ovule of *Magnolia grandiflora* at time of flowering.

OVULE AND EMBRYO SAC.—Development of the ovule in this plant is essentially the same as that in *M. umbrella* Desr. [*M. tripetala* L.] described by GRAY (9). Figure 1 is a longitudinal section of an ovule of *M. grandiflora* at the time of opening of the flower bud. The single vascular strand of the ovule is seen to pass unbranched throughout the entire length of the raphe, and then ends in the chalazal region of the ovule in short branches, one of which is directed toward the embryo sac. This condition is retained during the entire development of the seed.

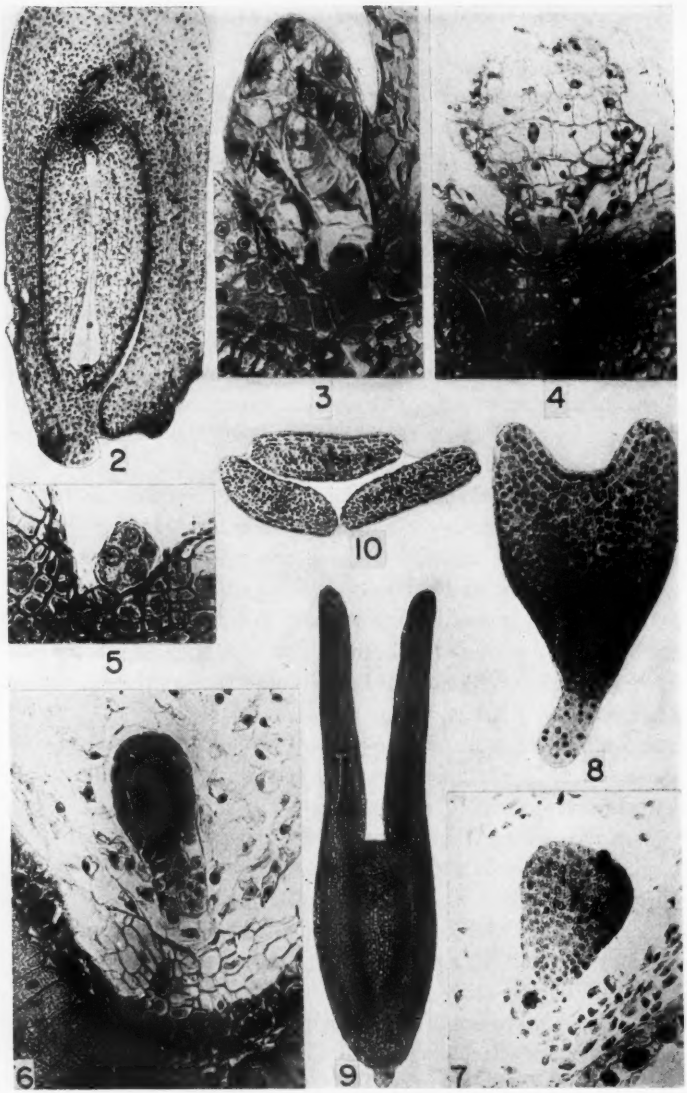
The mature embryo sac is long and slender, but enlarges into a club-shaped expansion at the micropylar end and also enlarges slightly at the chalazal end. It is extremely difficult to fix properly owing to the fact that it is surrounded by a considerable mass of nucellar tissue. It is circular in cross section, measuring about 110 μ in diameter at the largest portion of the club-shaped expansion, about 35 μ in diameter at the chalazal end, and is approximately 650 μ in length. The three antipodal nuclei are situated at the extreme base of the sac, the two polar nuclei occupy a position about one-fourth the length of the sac from the egg apparatus, while the egg and two synergids are at the apex of the sac.

ENDOSPERM.—The endosperm nucleus, either before or after fusion with the sperm nucleus, moves to a position even closer to the egg apparatus, and it is in this region that the endosperm starts its development. The early divisions of the endosperm nucleus are very rapid and the number of free nuclei formed is comparatively small. They occupy only the expanded end of the embryo sac. Wall formation occurs shortly after the appearance of these free nuclei and the endosperm appears as a globular mass of tissue, almost obscuring the fertilized egg (fig. 3). At this time the ovule begins to increase in size and the nucellus begins to disorganize, especially in the region of the growing endosperm. At first the endosperm is a rather compact mass of tissue (figs. 3, 4), the cells being thin-walled and relatively large. About three or four weeks after the external flower parts have fallen away the endosperm enlarges enormously, the cells becoming very much larger, and the tissue resorbing all of the nucellus except the small mass on which the embryo rests and a thin sheet immediately under the inner integument. At this time the endosperm is very watery and extremely difficult to fix on a slide. In material collected about the middle of August, however, the endosperm is firm in texture and is beginning to assume the character of the endosperm of the mature seed.

According to EVANS (**8**), the cells of the endosperm of the mature seed are rich in protein reserve bodies and small globules of oil, but contain no starch.

EMBRYO.—The fertilized egg remains dormant for a considerable length of time while the endosperm is developing rapidly. Ovules collected about two weeks after the flower had opened show the fertilized egg undivided, while the endosperm is a considerable mass of tissue (fig. 3). An embryo in the two-celled stage was not observed, but a three-celled embryo is shown in figure 4. The two lower cells of this embryo are in contact with the nucellus, while the third cell, which is larger than either of the two lower ones, is on top of them. It is obvious, therefore, that the first division is transverse and the second division is in the basal nucleus and perpendicular to the first. The somewhat later division of the upper nucleus is also perpendicular to the first division, and an almost spherical four-celled embryo results (fig. 5).

The divisions immediately following are comparatively slow and



FIGS. 2–10.—*Magnolia grandiflora*. Fig. 2, median longisection of ovule at time of flowering. Fig. 3, fertilized egg, cellular endosperm, and partially resorbed nucellus. Fig. 4, three-celled embryo and cellular endosperm. Fig. 5, four-celled embryo. Fig. 6, club-shaped embryo showing more vacuolated suspensor cells. Fig. 7, flat-topped embryo. Fig. 8, slightly older embryo showing primordia of cotyledons. Fig. 9, longisection of mature embryo. Fig. 10, transection through cotyledons of tricotyledonous embryo.

are probably not so uniform in their direction. About four weeks after the first division of the fertilized egg, which is about six weeks after pollination, the embryo is a slightly club-shaped mass of entirely undifferentiated cells, about ten cells high and several cells in thickness throughout.

Cell divisions become more frequent at this time, especially in the upper half of the embryo, and this portion bulges slightly in all directions, becoming hemispherical on the top and sides and tapering gradually into the massive stalk below (fig. 6). This stalk becomes a massive suspensor several cells in thickness. Cell divisions continue to be very frequent in the embryo and may be seen to take place in all planes, the whole structure becoming considerably larger, although still retaining the same shape. As yet there is no specialization of any of the cells in the body of the embryo, all of them being about the same size and containing large, spherical nuclei and dense cytoplasm. In the part that is to become a suspensor, however, the cells are somewhat larger and the cytoplasm less dense.

Material fixed about the middle of August shows a flattening of the distal end of the embryo (fig. 7). This condition is not retained long, for soon the rudiments of the cotyledons make their appearance (fig. 8). Both start at the same time and are exactly the same size. Embryos which were dissected out and examined showed no indication of a crescentic ridge from which the cotyledons might arise, but each cotyledon was seen to be an independent structure from the very beginning of its existence. They grow rather rapidly and it is a matter of only a few days until they are fairly elongated structures. Occasional embryos with three cotyledons were found, in which case the three cotyledons are equidistant from one another as in the normal type with two cotyledons.

While the cotyledons are growing both in length and thickness, numerous cell divisions may be seen in the body of the embryo in all planes. At the same time, the primordial cells of the primary vascular tissue are elongating, strands passing into each cotyledon from the hypocotyl. Growth in the whole embryo is rapid from this time on, although the cells in the massive suspensor have ceased dividing. Before the embryo has reached its full length the radicle may be distinguished and the plerome is well established, traversing the entire distance from radicle to cotyledons.

The embryo (fig. 9) is mature by about the middle of September. It is approximately 1 mm. in length. The plerome of the hypocotyl sends three branches into each cotyledon, and these are connected at various intervals by anastomosing strands of elongated cells. In cross section the cotyledons are slightly curved, their concave surfaces facing each other. The distal end of the hypocotyl between the cotyledons is flat in most cases but sometimes bulges slightly, and the protoplasm of the cells in this region is more compact than in the other cells of the embryo. The embryo remains attached to the massive suspensor, which still rests on the remains of the nucellus at the micropylar end of the seed. A root cap is distinguishable between the suspensor and the growing point of the radicle.

CIMICIFUGA RACEMOSA

Cimicifuga racemosa grows in rich woods throughout the eastern United States from Maine to Georgia, and as far west as Wisconsin. It reaches an altitude up to 4000 feet in the mountains of western North Carolina. Material was collected during the months of June, July, and August in 1934 and 1935 in Greenville County, South Carolina, and Brevard County, North Carolina.

The plants are rather conspicuous, with large, ternately compound leaves and long flowering stems, up to 10 feet in height. The inflorescence is a raceme up to 2 feet in length. The flowers consist of four or five petaloid sepals which fall away soon after the flower opens, several small clawed petals, numerous stamens, and usually a single pistil, although occasionally flowers with two or three pistils have been found. Flowering takes place from May to August in North and South Carolina, depending upon the altitude at which the plants are growing. The fruit is a follicle containing eight to twelve flattened seeds packed in two horizontal rows in the pod.

OVULE AND EMBRYO SAC.—The anatropous ovules lie in two horizontal rows in the pistil. Sections fixed about three weeks before the opening of the flower bud show the primordia of a pair of ovules as two protuberances of homogeneous tissue, directed obliquely from each other (fig. 11*A*). Unequal growth causes the ovular primordia to bend, and at the same time the inner integument makes its appearance. It appears as a collar-like ridge, de-

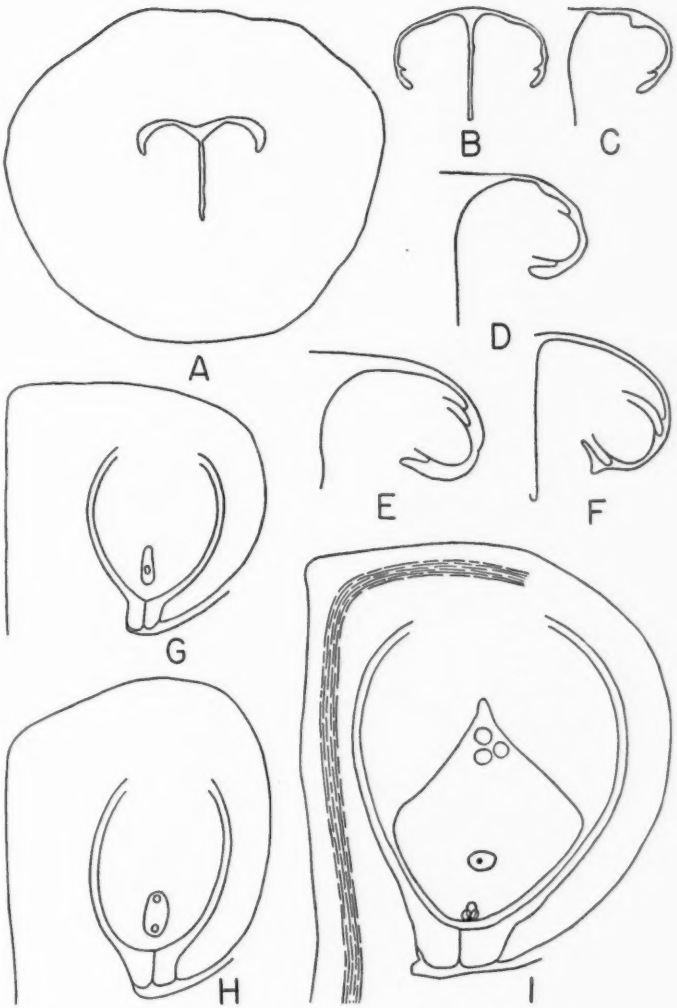
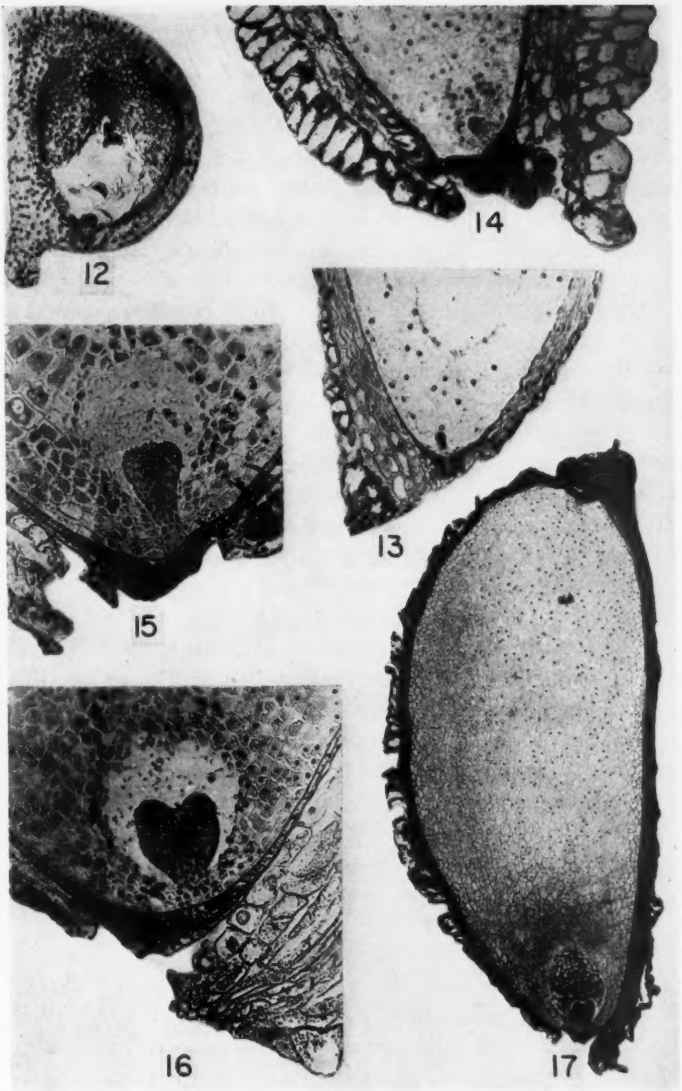


FIG. 11.—*A–I.* Development of anatropous ovule of *Cimicifuga racemosa*

veloping first on the under side of the nucellus (fig. 11*B*) and finally completely encircling it (fig. 11*C*, *D*). It is two layers of cells in thickness at the beginning and retains this condition during its entire existence, except in the part that surrounds the micropyle. Shortly after the appearance of the inner integument, the outer integument appears (fig. 11*D*, *E*). It is also two layers of cells in thickness at first but soon begins to thicken as a result of tangential divisions. The ovule continues to bend until finally it becomes entirely inverted (fig. 11*F*, *G*), during which time the inner integument grows completely down over the nucellus, forming the micropyle. In the micropylar region the inner integument becomes several layers of cells thick. The outer integument never quite reaches the micropyle. When the ovule is mature there is a single vascular strand traversing the raphe and ending blindly in the chalazal region (fig. 11*I*).

The mature embryo sac is practically rhombic in shape as seen in longitudinal section (figs. 11*I*, 12). One angle is situated at the micropylar end of the ovule and from the opposite angle there is a slight projection of the embryo sac into the nucellus. At the base of the sac are three prominent antipodal nuclei, a little above the center is a large fusion nucleus, and at the micropylar end is the egg apparatus, consisting of an egg and two synergids. At its greatest length the embryo sac is about 300 μ, and approximately 250 μ at its greatest width.

ENDOSPERM.—Division of the primary endosperm nucleus occurs soon after fertilization. The nuclei of the endosperm undergo a period of repeated, simultaneous, free divisions, and eventually are distributed in the parietal layer of cytoplasm of the embryo sac, as a single layer. While this parietal layer of free nuclei is forming, the nucellus is rapidly resorbed and the ovule is increasing in size. The nuclei of the endosperm are much larger than those of the developing embryo, and each contains three to five nucleoli. When the embryo consists of about eight to ten cells the nuclei of the endosperm layer undergo a periclinal division, forming a second layer inside the first. Wall formation then occurs between the nuclei, not simultaneously, but beginning between the nuclei in the chalazal region and progressing toward the micropylar end. Additional peri-



FIGS. 12–17.—*Cimicifuga racemosa*. Fig. 12, median longisection of ovule at time of flowering. Fig. 13, undifferentiated embryo and endosperm that has not filled embryo sac. Fig. 14, slightly club-shaped embryo with vacuolated suspensor cells; endosperm completely filling embryo sac. Fig. 15, flat-topped embryo surrounded by partially digested endosperm. Fig. 16, slightly older embryo showing primordia of cotyledons. Fig. 17, median longisection of mature seed showing embryo surrounded by partially digested endosperm.

clinal divisions take place in rapid succession and very soon the endosperm is a solid mass of tissue within the integuments, the nucellus having been completely resorbed. The endosperm is very watery at this time, but at about the time the club-shaped end of the embryo begins to enlarge (fig. 14) it is much firmer and the cells stain more heavily. Mitotic figures may still be seen in the part immediately surrounding the embryo. As the embryo enlarges the endosperm cells surrounding it appear to be partially digested. In the preparation of sections of ovules in later stages of development the walls of these partially digested endosperm cells do not stain with haematoxylin, thus giving the appearance of a cavity between the embryo and the undigested endosperm (figs. 15, 16). In sections stained with alcoholic safranin, however, the walls of these partially digested endosperm cells stain heavily, showing it to be a globular mass of tissue surrounding the embryo (fig. 17). The endosperm of the mature seed is oily in texture, the cells containing numerous small globules of oil.

EMBRYO.—The endosperm is still in the free nuclear condition when the first division of the fertilized egg occurs. The nucellus in the micropylar region of the ovule may be completely resorbed by the time the first division takes place, in which case the embryo is situated next to the inner integument, very close to the micropyle; or there may be a small amount of nucellar tissue still present between the young embryo and the integument. The direction of the first division of the fertilized egg is transverse, the basal cell of the two-celled embryo exceeding the other in size. The plane of division of the basal cell is perpendicular to the first, while the apical cell is divided by an oblique wall.

Divisions from this time on appear to be irregular and comparatively slow. By the time the endosperm has completely filled the embryo sac and has resorbed all of the nucellus, the embryo is a club-shaped mass of cells, about ten cells in length, two cells in thickness at the base, and four cells across at the thickest part of the club-shaped expansion (fig. 14). Although the expanded apical end of the embryo contains many more cells than the basal portion, it is not proportionately larger, since the cells of the basal part are a great deal larger than those of the upper part. These basal cells

form a suspensor in the mature embryo. Cell divisions become more frequent at this time, there being apparently no regularity to their plane, since mitoses in various directions may be observed. The only differentiation is found in the suspensor, the cytoplasm in the cells of this structure being more vacuolated than in the other cells of the embryo. Next, the cells of the club-shaped portion become enlarged, thus increasing the size of the embryo. Very soon thereafter the hemispherical end of the embryo becomes flattened across the top (fig. 15). The embryo enlarges still further and the cells in the central region become elongated, forming the primordium of the plerome. The primordia of the cotyledons make their appearance as small protuberances on opposite sides of the flat-topped structure (fig. 16). Embryos of this age which were dissected out and examined showed the cotyledons to be entirely separate and independent structures, there being no indication of a crescentic ridge from which they might arise. They never attain much prominence until germination occurs, and in the ripe seed appear as two small knobs of tissue (fig. 17). There is no indication of a differentiation of cells of either of the growing points of the embryo in the mature seed. The body of the embryo is entirely symmetrical, although the suspensor is slightly curved in nearly all cases.

Historical review

Magnoliaceae

The embryology of *Magnolia virginiana* L., described by Maneval (12), corresponds in all essential details to what has been found for *M. grandiflora*. There is built up by irregular cell divisions a pear-shaped mass of tissue, at the base of which is a massive suspensor. The cotyledons then appear simultaneously as two small and independent protuberances at the distal end of the embryo. In the development of the endosperm of this plant, as described by Maneval, the first division of the primary endosperm nucleus results in a wall between the daughter nuclei, and both of these nuclei participate in the formation of the endosperm. This is not true for *M. grandiflora*, for while formation occurs very early, there is a brief period when free endosperm nuclei are present.

STRASBURGER (18) published a figure of a very young embryo of *Drimys winteri* Forst. showing a massive suspensor. In the vicinity of the embryo free endosperm nuclei are present. Later stages were not described.

RANUNCULACEAE

OSTERWALDER (15) described the early divisions of the cells of the embryo of *Aconitum napellus* L. as being very irregular, and yet by the time the 28–34-celled stage is reached a dermatogen is differentiated. Such an early differentiation has not been described for any other Ranalean embryo and was not found in either *Magnolia grandiflora* or *Cimicifuga racemosa*. In the further development the cotyledons appear as two small, independent protuberances from the distal end of the embryo, which is attached to the nucellus by means of a short massive suspensor. The endosperm at first consists of free nuclei.

COULTER (7) described the early development of the embryos of *Ranunculus septentrionalis* Poir. and *R. multifidus* Pursh. In both plants the early divisions of the fertilized egg are irregular and a massive suspensor is developed. The later stages were not studied.

MOTTIER (14) studied the embryology of several members of the Ranunculaceae. The young embryos of *Actaea alba* (L.) Mill. and *Delphinium tricorne* Michx., before development of the cotyledons, are described as being typically pear-shaped with a massive suspensor. A crescentic ridge is described as forming at the distal end of the embryo and then bifurcating to form the two cotyledons. MOTTIER has drawn several series of transverse sections through various levels of the embryos to show a deeper cleft between the cotyledons on one side than on the other. That these apparently asymmetrical figures may be an incorrect interpretation due to oblique sectioning of the embryos is shown by figure 18, which is a series of transverse sections through an embryo of *Magnolia grandiflora* which had been carefully examined with a binocular prior to imbedding and found to be perfectly symmetrical, the clefts between the cotyledons being of equal depth. The embryo, however, was sectioned at a slightly oblique angle and the sections correspond to those made by MOTTIER for both *Actaea* and *Delphinium*. Further-

more, this interpretation (that is, a crescentic ridge from which the cotyledons arise) is not comparable with what has been observed in *Cimicifuga racemosa*, for in this plant the cotyledons make their appearance as two entirely independent structures.

In *Aquilegia canadensis* L. the anomalous cotyledonary primordium is said to exist only as a trace, since it bifurcates soon after being laid down, while in *Anemonella thalictroides* (L.) Spach. no such crescentic ridge was observed, the two cotyledons being independent at the beginning.

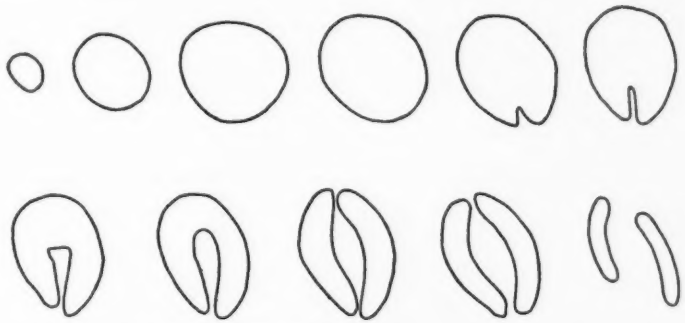


FIG. 18.—Series of transections through mature embryo of *Magnolia grandiflora*

The morphology of the embryo and seedling of *Ranunculus ficaria* L., which is peculiar in having only one cotyledon, has been the source of numerous investigations. WOLTER (19) described the early cell divisions in the formation of the embryo as being very irregular, and at the time the fruit fell there was no plerome, periblem, or dermatogen differentiated. METCALFE (13), in an account of the seedling anatomy of *R. ficaria*, published several figures of the embryo in various stages of development after germination of the seed. These show a suspensor, the single cotyledon, that is at least two cells in thickness all the way to the base, a small "hump" of tissue opposite the cotyledon occupying a position that would normally be the position of the second cotyledon, and between these two structures the apical growing point. METCALFE inter-

preted the small hump of tissue as an aborted cotyledon, while SARGANT (16) regarded the large cotyledon as the result of the fusion of two cotyledons. METCALFE'S view is accepted here.

In all cases described for the Ranunculaceae, free endosperm nuclei are formed in the early stages.

BERBERIDACEAE

LEWIS (10) states that in *Podophyllum peltatum* L., *Caulophyllum thalictroides* Michx., and *Jeffersonia diphylla* (L.) Pers. a crescentic ridge forms at the distal end of the embryo and then bifurcates to form the two cotyledons. BUTTERS (1), however, with the use of models and drawings, has shown that the cotyledons in *Caulophyllum* do not arise so but appear as two moundlike outgrowths occupying opposite sides of the margin of the distal end of the embryo. And CLARK (2), with the use of models, drawings, and photographs of actual embryos, has shown that the two cotyledons in *Podophyllum* have their origin as two independent protuberances at the distal end of the embryo. By intercalary growth a cotyledonary tube is developed. In the light of these more recent investigations it seems desirable that further studies be made on the embryology of *Jeffersonia* to test the validity of the assertion that the cotyledons arise as described by LEWIS.

BUTTERS and CLARK agree with LEWIS that in the early stages the embryos in both *Caulophyllum* and *Podophyllum* are entirely undifferentiated bodies, with rather large, massive suspensors. In both plants the endosperm consists at first of free nuclei.

NYMPHAEACEAE

COOK (6) has pointed out that embryo development in this family is subject to considerable variation, and that variation may occur even within a species, unless there has been confusion in the identification of closely related forms. In any event it is evident that there are certain features common to the group as a whole. LYON (11) and YORK (20) have shown that a suspensor is absent in *Nelumbo lutea* (Willd.) Pers. and that the two cotyledonary lobes arise from a common primordium, thus suggesting the presence of a single,

bilobed cotyledon, which is interpreted as such by these workers. Cook (4) described the embryos of *Nymphaea advena* Ait. [*Nymphozanthus advena* (Ait.) Fernald] and *Castalia odorata* (Dryand.) Woodv. & Wood. [*Nymphaea odorata* Dryand.] as being similar to *Nelumbo lutea* in development, except that in *Nymphozanthus* a "latent" suspensor is developed. Conard (3), however, has shown that a filamentous suspensor may be present in *Nymphaea odorata*, as well as in *N. lotus* (L.) Willd. In a later paper Cook (6) also reported the presence of a filamentous suspensor in *Nymphaea ampla* (Salisb.) DC. and *N. pubescens* Willd., and that such a suspensor may or may not be present in *N. odorata*. He confirmed his previous work as to the presence of a cotyledonary ridge in *Nymphaea odorata* and *Nymphozanthus advena*, as well as that of Lyon and York as to the presence of the same structure in *Nelumbo lutea*.

It has been shown by Cook (4) that in *Nymphaea odorata* and *Nymphozanthus advena* a wall is formed across the embryo sac following the first division of the primary endosperm nucleus, thus producing a cellular endosperm from the beginning. York (20) has noted the same thing in *Nelumbo lutea*. In *Cabomba piauhiensis* and *Brasenia purpurea*, however, a few free endosperm nuclei are at first formed (5).

Ceratophyllaceae

Development of the embryo of *Ceratophyllum submersum* L., described by Strasburger (17), is strikingly similar to that of *Nelumbo lutea*. A suspensor is lacking and the cotyledonary lobes arise from a common primordium, although there is not so great a difference in the depth of the clefts between the lobes as in *Nelumbo*. A wall divides the embryo sac following the first division of the endosperm nucleus, thus producing a cellular endosperm from the beginning.

Discussion

The manner of development of the various members of the Ranales that have been described is remarkably similar in certain respects and very different in others. The early undifferentiated mass of cells is a character that seems common to all Ranalean

embryos and very closely resembles the condition found in many monocotyledons. Furthermore, Ranalean embryos seem to conform to a general type in having very irregular cell divisions in the early stages and very late differentiation of tissues.

In all cases described for the Magnoliaceae, Berberidaceae, and Ranunculaceae, a suspensor is formed early in the development of the embryo. Although MOTTIER has described the presence of a crescentic ridge of tissue from which the cotyledons arise in *Actaea alba* and *Delphinium tricorne*, this has not been found in other members of the Ranunculaceae, and there is evidence for doubt as to its presence in these two plants. Such a structure is not present in the members of the Magnoliaceae and Berberidaceae that have been investigated, the cotyledons arising as independent structures. In general, then, it may be said that the embryo development, as well as that of the endosperm, in the Magnoliaceae, Berberidaceae, and Ranunculaceae is very similar, but is different from that of the Nymphaeaceae and Ceratophyllaceae. The embryo and endosperm development of *Magnolia grandiflora* and *Cimicifuga racemosa* further strengthens this view.

While there is considerable variation within the Nymphaeaceae with respect to the presence or absence of a suspensor, in other respects the embryology of the group is very uniform and is also very similar to the embryology of the Ceratophyllaceae. The most striking similarity in the embryo development of these two families is the manner of origin of the cotyledonary lobes (from a common primordium), which has been interpreted by some workers (LYON, YORK, COOK) as being a single bilobed cotyledon, and by others (STRASBURGER, CONARD) as being two cotyledons.

It is of particular interest that the endosperm of some of the plants of these two families is cellular from the beginning and resembles the endosperm development of the Aroids in this respect.

Whether the similarities that exist between the embryology of the Nymphaeaceae and Ceratophyllaceae, and the dissimilarities that exist between these two families and the Magnoliaceae, Ranunculaceae, and Berberidaceae, are sufficient justification for suggesting that the Nymphaeaceae and Ceratophyllaceae be placed in the Monocotyledonae, as has been done by a number of workers for the

Nymphaeaceae, is not within the scope of this paper, but they do seem highly significant.

Summary

1. The anatropous ovule of *Magnolia grandiflora* develops in a manner similar to that described by GRAY for *M. tripetala*.

2. The embryo sac of *M. grandiflora* is long and slender, and terminates in a club-shaped expansion at the micropylar end. It contains the usual number of eight nuclei.

3. The endosperm of *M. grandiflora* is cellular very early in its development and finally comes to be a massive structure, containing oil but no starch.

4. The embryo of *M. grandiflora* at first is a mass of undifferentiated cells with a massive suspensor. It becomes pear-shaped by irregular cell divisions. The cotyledons arise from the distal end as independent structures.

5. The development of the anatropous ovule of *Cimicifuga racemosa* shows no marked peculiarity.

6. The embryo sac of *C. racemosa* is of the usual type, having eight nuclei. It is rhombic in shape as seen in longitudinal section.

7. The endosperm of *C. racemosa* in its early stages consists of free nuclei lining the wall of the embryo sac. Wall formation occurs about these free nuclei and the endosperm finally fills the embryo sac. It contains oil but no starch.

8. The embryo of *C. racemosa* is similar to that of *M. grandiflora* in its development, consisting at first of a globular mass of undifferentiated cells. The cotyledons arise from the distal end as independent structures. There is a massive suspensor at the base of the embryo.

9. Development of the embryos of *M. grandiflora* and *Cimicifuga racemosa* corresponds to what has been found in other species of the Magnoliaceae, Ranunculaceae, and also of the Berberidaceae.

10. The embryology of the Ranales as a whole seems to conform to a general type in the early stages, closely resembling the condition found in many monocotyledons; in its later stages it is thoroughly dicotyledonous in the Magnoliaceae, Berberidaceae, and Ranunculaceae.

11. There seems to be some basis for the suggestion that the Nymphaeaceae and Ceratophyllaceae be placed in the Monocotyledonae.

The writer wishes to thank Professor F. K. BUTTERS, of the University of Minnesota, who suggested the problem and gave much valuable counsel during the course of the investigation.

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RELATION OF SOIL TEMPERATURE AND NUTRITION TO THE RESISTANCE OF TOBACCO TO THIELAVIA BASICOLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 494

FRANCES LOUISE JEWETT

(WITH THIRTY-SEVEN FIGURES)

Introduction

Of the diseases which have been studied from the standpoint of the effect of environment on resistance and susceptibility, black root rot of tobacco caused by the fungus *Thielavia basicola* Zopf has received much attention. Field and greenhouse experiments have shown that soil reaction and soil temperature are the most important external conditions determining the severity of the disease. The field experiments of BRIGGS (7), in which he studied the amount of acidity as measured by the lime requirement method, and the later work of ANDERSON (1), DORAN (12), and others in which they measured the intensity of the acidity by determining the pH of the soil, have shown the importance of soil reaction. ANDERSON reports, however, that the range of pH within which tobacco does not become infected with *Thielavia* may vary slightly with the temperature. JOHNSON and HARTMAN (15) tested the effect of various factors, including soil reaction, and concluded that soil temperature is the most important single controlling factor in determining the severity of black root rot of tobacco. Low temperatures, from 17° to 23° C., are the most favorable for development of the disease, while at 29°–30° C. there is very little infection. At 32° C. there is practically no infection, even in the most susceptible varieties of tobacco.

The reasons for this insusceptibility of susceptible varieties at high temperatures are not clear. CONANT (10) reported a distinct correlation between the resistance of the plant and its ability to form cork in advance of the fungus and beneath lesions. The most resistant varieties formed cork readily at low temperatures (20° C.);

the very susceptible varieties formed no cork at low temperatures, but formed it abundantly at the high (30° C.). CONANT states that resistance to *Thielavia* can be accurately measured by the rapidity with which the plant can form cork beneath a lesion.

The first purpose of the present study was to determine the effect of changes in nitrogen nutrition, in conjunction with changes in soil temperature, on the relative resistance of five varieties of tobacco to *Thielavia basicola*. The second purpose was to examine microscopically the roots of these varieties to see the effects of these changes in nutrition and temperature on cork formation. It was also hoped that it could be determined whether a corky layer such as described by CONANT is laid down in advance of or subsequent to fungal invasion, after the initial infection.

Materials and methods

The varieties of tobacco used were: Ordinary White Burley, susceptible; Resistant White Burley, resistant; Connecticut Havana 38, resistant; Havana 142, very resistant; and Xanthia, extremely resistant.

Two cultures of *Thielavia* from the University of Wisconsin, an Italian strain and a Wisconsin strain, proved ineffective in producing infection in tobacco under the conditions of the experiment. New cultures were obtained by isolation from plants grown in infected soil brought from the experimental farm of the University of Wisconsin at Madison. These cultures were used successfully to obtain infection.

The experiments were carried out in the greenhouses of the University of Chicago, using the soil-nutrient temperature tank as described by LINK (19). The plants were grown from seed in sterile soil; when they had reached the six or seven leaf stage, two plants were transferred to each pot of the temperature tank, in which sterile quartz sand was used instead of soil. The temperatures used in all but one series were 28°–30° C. for one set of tanks and 18°–20° C. for the other. In one series, grown during extremely hot summer weather, 24°–26° C. was the lowest soil temperature attainable in the tank.

For each temperature, half of the tanks were given nutrient solu-

tion containing nitrate nitrogen, and the other half, solution lacking the nitrogen. The composition of the nutrient solution given in partial volume molecular concentration is as follows:

SOLUTION	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	$CaCl_2$
Plus nitrate.....	0.0090	0.0045	0.0045	.....
Minus nitrate.....	.....	0.0045	0.0045	0.0090

The pH of the solutions made with distilled water is approximately 5.7 and no adjustment was made. Five hundred cc. of the solution was given to each pot of +N plants three times a week, while the −N plants received 500 cc. twice a week.

Five series of plants were grown. In series I and II the plants were supplied with nutrient solution a month before inoculating with pure cultures of *Thielavia*. By this time the external characteristics of the +N and −N plants were evident, and a nitrate test of the −N plants showed an absence of nitrate nitrogen in the tissues. In these series the pH of the sand was kept between pH 5.6 and 5.9 by watering with acidified tap water. Within this range is the zone in which environmental factors other than pH are limiting, according to ANDERSON and others (1). Plants of series III–V were given nutrient solutions and inoculated from two to three days after placing in the tanks. These series were watered with non-acidified water at regular intervals.

The inoculum for series I–III and V was prepared by growing the fungus in Erlenmeyer flasks on potato dextrose agar. After five to twelve days' incubation, when examination showed an abundance of endoconidia, suspensions of the spores were made by pouring sterile tap water into the flasks, shaking vigorously, and scraping the mat with a sterile inoculating needle. Inoculation was accomplished by clearing away the sand around the plant and pouring some of the suspension directly on the roots. The rest of the suspension was thoroughly mixed with the surrounding sand. In the case of series IV a small amount of infected soil from the field was used as inoculum. This was thoroughly mixed with the sand and was very effective in producing infection.

A set of plants of each temperature and plane of nutrition was kept uninoculated as controls. During the growing period the roots

of the controls of series II and V were wounded by pricking with a fine needle, in order to examine the type of wound reaction shown under the different conditions in the absence of the fungus. In the case of the −N plants, the roots are so small that the wound was made at the base of the stem, or crown, which is below the surface of the soil. Since this region is also attacked by the fungus, it is the region studied in most of the −N plants.

At the end of six weeks the plants were removed from the tanks and measured for total height and relative growth of the roots and tops. The roots were carefully washed and examined microscopically for evidences of infection with *Thielavia*, the chief evidence being the presence of the characteristic chlamydospores.

Material from the control and infected plants was killed and fixed in formal acetic-alcohol, imbedded in paraffin, sectioned on the rotary microtome at 10–12 μ, and stained with Flemming's triple stain.

Results

Greenhouse experiments

Table 1 summarizes the results obtained in the tank experiments in the greenhouse. Since no record, except failure to obtain infection, was kept of series III, this series was not included in the table. This summary indicates that between the +N and −N plants there is no difference in resistance and susceptibility which is either sufficiently striking or sufficiently consistent to be evident from a limited number of small scale experiments.

Between the high temperature and the low there is a difference both in the number of varieties affected and in the severity of the infection. At the low temperature the number of varieties showing infection is greater, since at this temperature the more resistant varieties immune at high temperatures become infected. The infection is also more severe at the lower temperature, as can be seen from the greater degree of decay of the roots and the greater stunting of the plant as a whole.

Although the number of varieties affected increases with a decrease in temperature, the order of severity of infection parallels the order in which the five varieties used are rated as to suscepti-

bility. This is true for all temperatures and for both +N and −N plants. The combined effect of temperature and the genetic constitution of the variety is seen in series I, in which the fungus culture was very weakly aggressive. The only undoubted case of infection occurred in the most susceptible variety at the low temperature.

The results of these experiments are in agreement with the general finding of others, that of the environmental factors tested, soil temperature is the most important one influencing the expression of

TABLE 1

COMPARATIVE INFECTIONS OF FIVE VARIETIES OF TOBACCO UNDER +N AND −N CONDITIONS AT DIFFERENT TEMPERATURES (IN DEGREES C.)

Variety	Series I				Series II				Series IV				Series V			
	+N		−N		+N		−N		+N		−N		+N		−N	
	18–20	28–30	18–20	28–30	18–20	28–30	18–20	28–30	18–20	28–30	18–20	28–30	18–20	28–30	18–20	28–30
Ordinary White Burley	+	−	+	−	−	−	−	−	+	+	+	−	+++	++	+++	++
Resistant White Burley	−	−	−	−	−	−	−	−	+	+	+	−	++	++	++	++
Havana 38	−	−	−	−	−	−	−	−	+	±	+	−	++	++	++	++
Havana 142	−	−	−	−	−	−	−	−	±	−	±	−	++	+	+	++
Xanthia	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	±

− = no infection; ± = slight infection; +, ++ = fairly heavy to heavy infection; +++ = severe infection.

resistance and susceptibility of varieties of tobacco to *Thielavia*. The results also show the importance of the breeding of resistant strains as a practical method of control, since even at the low temperatures most favorable for the disease, resistant varieties are not so severely affected as susceptible varieties, although they may not be completely immune.

MICROSCOPIC EXAMINATION OF ROOTS

UNWOUNDED CONTROLS.—At both the high temperature (28°–30° C.) and the low (18°–20° C.), a somewhat more regular and compact periderm was found in the +N control plants than in the −N ones. At both temperatures +N susceptible Ordinary White Burley

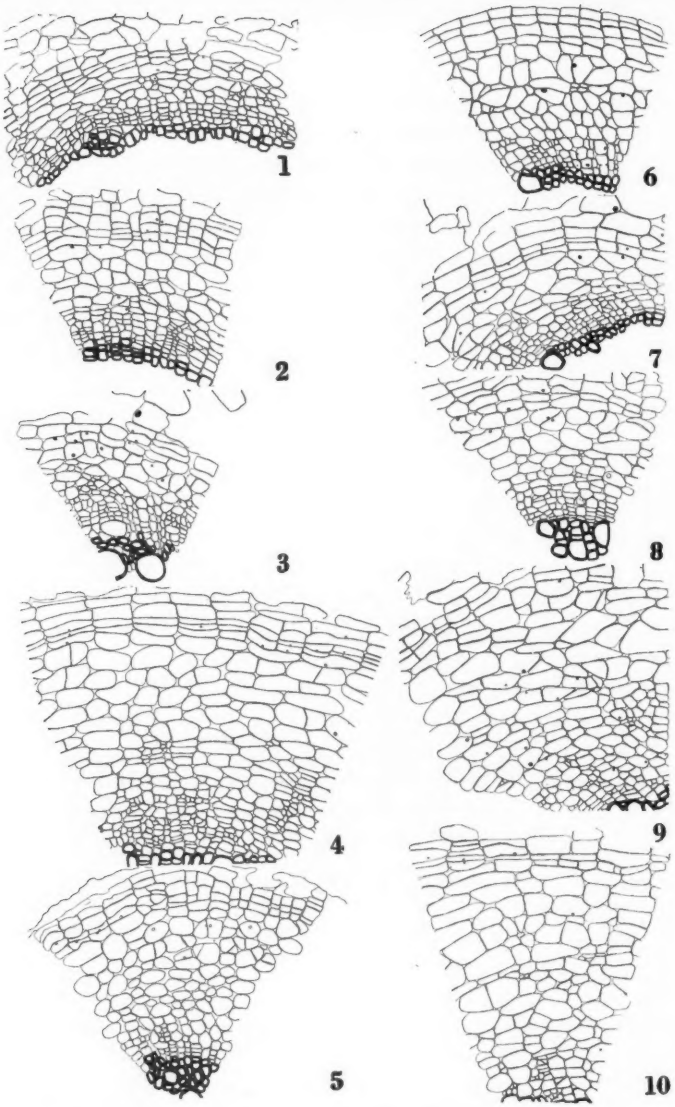
showed as well developed periderm as very resistant Xanthia. In all cases, however, the White Burley was much more severely infected than Xanthia, which remained almost entirely free of infection. The same was true of Resistant White Burley, which is more susceptible than Xanthia. At both temperatures and planes of nutrition the most extensive and continuous periderm was found in the roots of Havana 142, a very resistant variety although not so resistant as Xanthia.

At both temperatures very resistant Xanthia showed more regular periderm formation in the −N plants than did susceptible White Burley.

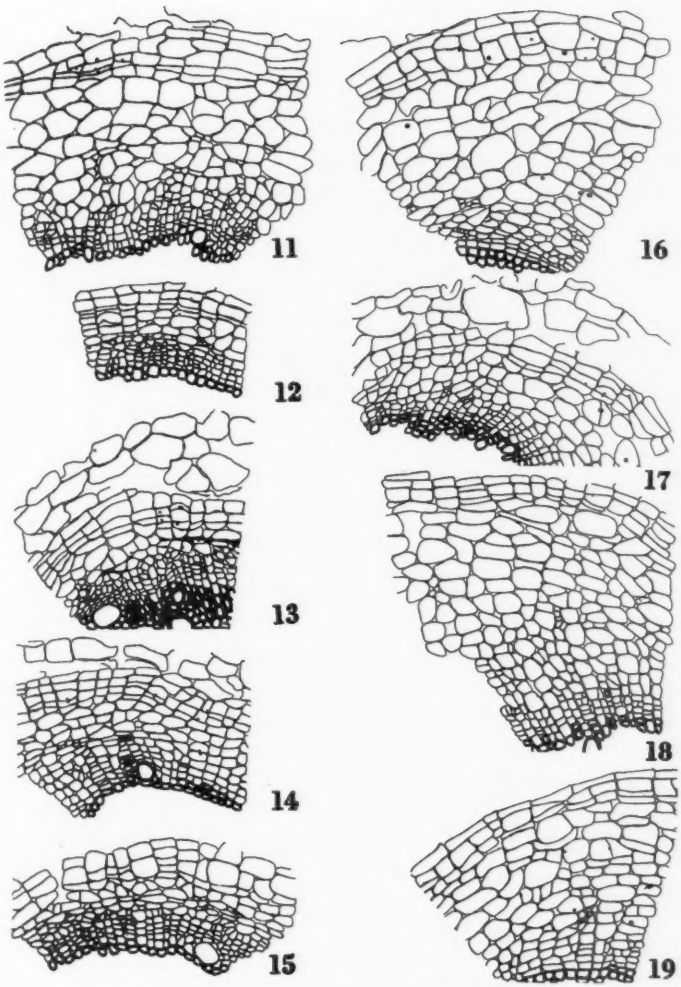
Havana 38, which is intermediate as to resistance, is the only one which showed more regular periderm formation at higher temperatures than at low. This was true for both +N and −N plants. Comparing Havana 38 with Xanthia, it was found that at the higher temperature +N Havana 38 showed slightly better developed periderm than +N Xanthia, while −N Havana 38 had a periderm which was at least as well developed as that of Xanthia. At the lower temperature Xanthia formed a more extensive periderm in both +N and −N plants. At both temperatures Havana 38 was much more severely affected than Xanthia.

Figures 1–19 show portions of the roots of the different varieties studied, giving in each case the region of the best developed periderm noted. The development of a protective layer was by no means uniform, either in extent around the root or in depth and compactness of the periderm cells. Havana 142 showed the most regular development. The thickness of the walls of these cells and their reaction to stains indicated that they were not always very heavily suberized. Their efficiency as a factor in resistance is doubtful.

The base of the stem which is underground, and which may become as heavily infected as the roots, showed no regular protective periderm layer. The epidermal cells were rather heavily suberized, as were the subepidermal cells in some cases. In certain regions a subepidermal periderm had developed, although in many stems no very extensive periderm was formed at any point, nor was the periderm uniform around the whole stem. One side of the stem often



FIGS. 1–10.—Portions of cross sections of roots of control plants grown at 18°–20° C., showing comparative peripheral periderm development. Figs. 1–5, +N: 1, Xanthia; 2, Havana 142; 3, Havana 38; 4, Resistant White Burley; 5, Ordinary White Burley. Figs. 6–10, −N: 6, Xanthia; 7, Havana 142; 8, Havana 38; 9, Resistant White Burley; 10, Ordinary White Purley.



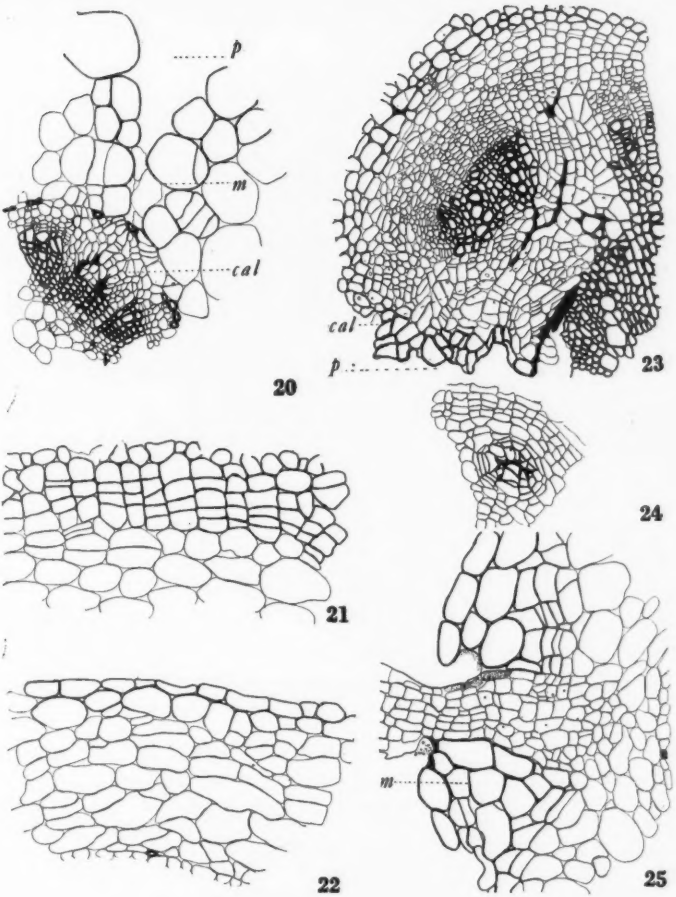
FIGS. 11–19.—Portions of cross sections of roots of control plants grown at 28°–30° C., showing comparative peripheral periderm development. Figs. 11–15, +N: 11, Xanthia; 12, Havana 142; 13, Havana 38; 14, Resistant White Burley; 15, Ordinary White Burley. Figs. 16–19, −N: 16, Xanthia; 17, Havana 38; 18, Resistant White Burley; 19, Ordinary White Burley.

showed a periderm of several cells in thickness, while the other side had only the suberized epidermal cells as protection (figs. 21, 22). So far as could be seen, the structure of the stem with respect to periderm formation was unchanged in relation to differences in temperature and nutrition. No marked differences were observed in the several varieties of tobacco used.

PROTECTION AT BASE OF BRANCH ROOTS.—Examination of the bases of branch roots emerging from both stems and roots showed no consistent formation of periderm as a protective layer in the areas exposed by the breaking through of the emerging root. In the case of secondary roots there was only the slightest evidence of the continuous peripheral cork layer described by CONANT (10). There were very definite reactions in the cortex of the stem in response to the injury made by the emerging adventitious root (fig. 25). The staining reaction indicated that the composition of the cells of the walls of the stem adjacent to the emerging root had changed to a substance staining like lignin or suberin. The walls of such cells also appeared somewhat thickened. Near the periphery of the stem there was some cell enlargement accompanying the change in nature of the cell wall. Very often cell divisions resulted in the formation of a weak periderm-like layer delimiting the wound made by the root emergence. Gum deposits were seen in the small pockets made by the destruction of the cells of the stem. There was apparently no correlation between the intensity of the reaction and the relative resistance of any variety examined. The reactions described were entirely comparable to the responses to other wounds in the cortex, a condition noted by BOYLE (5) in relation to emerging secondary roots in flax.

Examination of cross sections of control roots of as nearly comparable stages of development as possible showed that the amount of normal periderm formed by the five varieties of tobacco under the different conditions of the experiment could not be clearly correlated with the order of the rating of these varieties as to their susceptibility and non-susceptibility to *Thielavia*.

REACTIONS TO INJURY.—The most common response to injury was the change in constitution of cell walls in mechanically injured and infected areas. This change is evident from the reaction of the



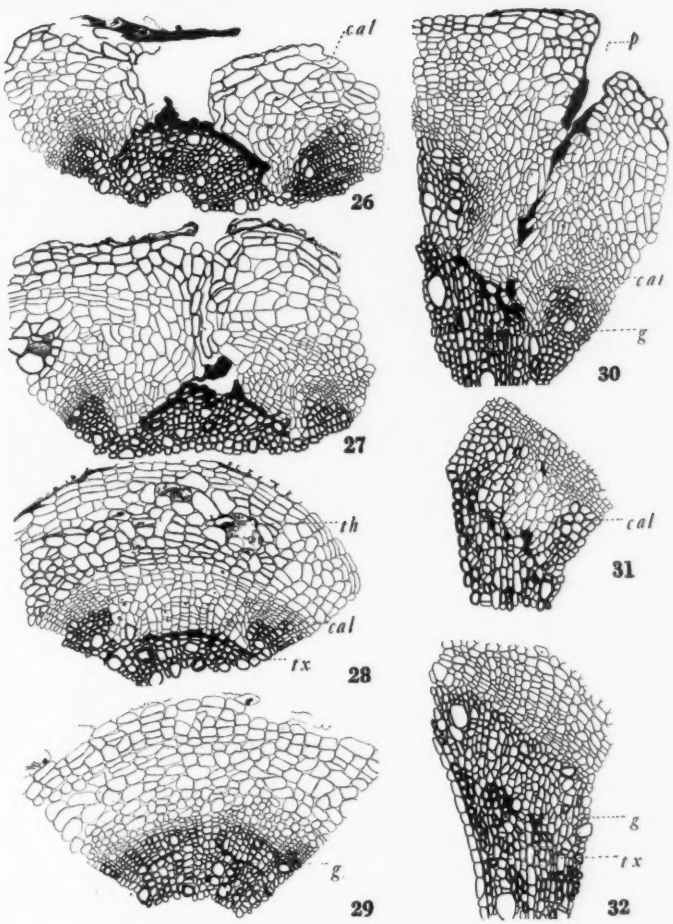
FIGS. 20–25.—Fig. 20, needle wound and reactions in stem, showing metacutinization of cortical cell walls, wound gum, cell divisions in cortex, and callus formation (*p*, path of needle; *m*, metacutinization; *cal*, callus); Havana 142, 18°–20° C., −N; control. Figs. 21, 22, portions of periphery of same cross section of stem base, fig. 21 showing subepidermal periderm on one side and fig. 22 lack of periderm on other side of stem; Havana 142, 18°–20° C., +N; control. Fig. 23, isolation of portions of xylem by callus development stimulated by injury with needle; cells bordering path of needle show metacutinization of walls, indicated by heavier lines; Ordinary White Burley, 18°–20° C., +N; control. Fig. 24, small wound in cortical region of root, showing deposit of gum surrounded by cells reacted by dividing; Ordinary White Burley, 18°–20° C., +N; control. Fig. 25, wound reactions around emerging adventitious root and showing evidence of cell divisions and wound gum formation; Havana 38, 24°–26° C., −N; control.

walls to stains. Cells whose walls stained orange in healthy roots, indicating cellulose with Flemming's triple stain, change to the red of lignified or suberized walls in injured regions. This change in constitution is comparable to the metacutinization of cell walls in injured areas described by KÜSTER (18). In control plants, cells bordering wounds of even very small extent show this reaction. In infected plants this response is found in cells adjacent to those containing the fungus as well as in the infected ones themselves.

Metacutinization is often accompanied by thickening of the wall of infected cells or of cells nearest the region destroyed by the fungus or by mechanical injury. Marked thickening of xylem elements is a characteristic response to injury (figs. 26–28, 32).

In addition to these changes in cell walls, deposits of a dark staining substance, apparently wound gum, are found in injured areas. Small wounds in the cortex result in the formation of pockets of gum surrounded by cells which have reacted by changes in the cell wall and by cell divisions (fig. 24). Similar pockets are found in injuries at the periphery of the root. Heavy gum deposits are seen in severely infected regions and in deep mechanical wounds. These deposits border the area of destroyed cells of the cortex and phloem, and are found on the face of the xylem where the cambium and some of the xylem elements have been destroyed (figs. 26, 27, 30). Gum in and between the xylem elements is one of the first wound reactions visible at a distance above or below the area actually destroyed (figs. 29, 32). In less severe mechanical injury and in infection this substance is seen as a coarsely granular material. In diseased roots it is often seen in the cells containing the fungus, where it appears as a granular coating on the hyphae (fig. 36).

Stimulation to cell division is a further reaction to injury. More or less regular divisions occur in the affected cells around some wounds (figs. 24, 33), but for the most part the divisions are irregular and do not result in the formation of a layer anatomically comparable to a compactly organized periderm. In deep lesions of both mechanical and fungal origin living cells bordering the destroyed areas are stimulated to callus formation (figs. 26–28, 30, 31). Involved in this formation are the cells of the cambium undestroyed at the edges of the wound and the parenchyma cells of the other



FIGS. 26–32.—Comparison of fungus injury with mechanically induced wound: Figs. 26–29, injury caused by *Thielavia basicola;* Havana 142, 18°–20° C., −N: fig. 26, region of greatest tissue destruction; wound gum on face of xylem and callus developing at edges (*cal*, callus). Chlamydospores of *Thielavia* in debris at periphery of root; fig. 27, region just beyond that shown in fig. 26; fig. 28, beyond fig. 27, approaching edge of region which had been destroyed (*th*, hyphae of *Thielavia*); metacutinization of cortical cell walls indicated by heavy lines; wound response in xylem seen in thickening of some xylem elements (*tx*); fig. 29, beyond region of tissue destruction; evidence of injury seen in wound gum (*g*) in xylem. Figs. 30–32, wound mechanically induced by pricking; Ordinary White Burley, 28°–30° C., +N; fig. 30, just above center of path of needle (*p*); fig. 31, region beyond that of fig. 30, approaching limits of the injury, but responses seen in callus and wound gum formation and thickening of xylem elements; fig. 32, beyond region of cell destruction; evidences of wound reaction in xylem in gum deposits and thickened xylem elements.

tissues bordering the injured region. Stimulation of the xylem parenchyma to division often results in the irregular development of the xylem due to the formation of areas of callus tissue isolating one part from the rest (fig. 23). This irregular development was seen in both types of injury, and was also mentioned by CONANT as one of the reactions to the invasion of *Thielavia*.

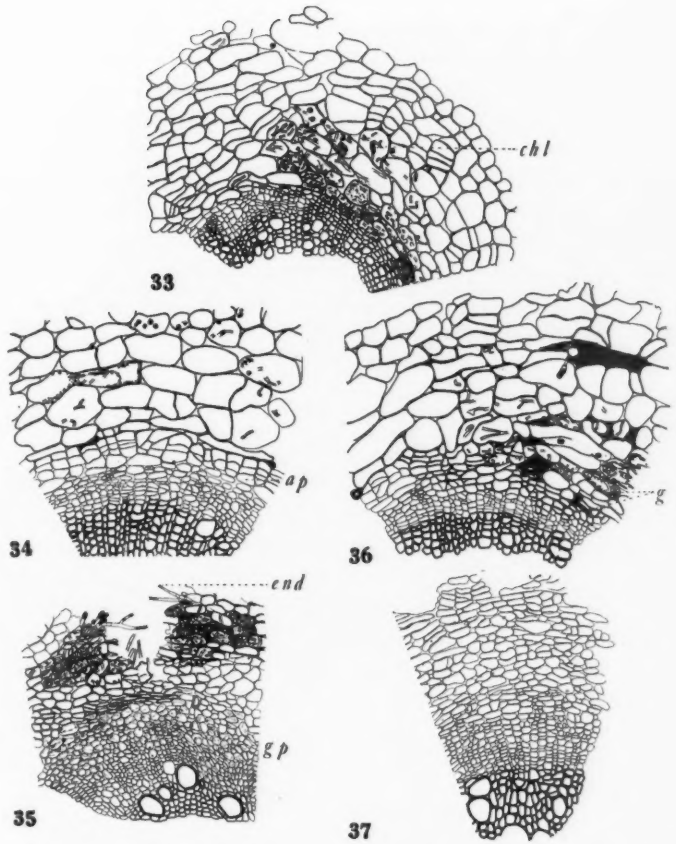
The callus tissue effectively heals the wound. This type of reaction to local injury was found in stems as well as in roots in the five varieties studied under the different conditions of the experiment (fig. 20). No differences were noted in amount or type of tissue formed which might be correlated with resistance and susceptibility.

One reaction seen in infected roots was not seen in those mechanically injured. Beneath heavily infected areas, the protoplasm was more granular and stained much more deeply than the protoplasm in similar cells in healthy areas of the same root (figs. 35, 37). This response to the fungus was found in these roots only when infection was rather severe. TISDALE (**26**) described a similar appearance in the cells of infected flax roots, and suggested that the change might be due to a chemical reaction brought about by the presence of the fungus.

These microscopic examinations of roots and crowns of tobacco plants infected with *Thielavia basicola* under the conditions of this experiment failed to corroborate the findings of CONANT with respect to periderm formation around lesions resulting from the presence of the fungus. A comparison of injuries made by *Thielavia* with those induced mechanically showed that the reactions to wounding were of the same type regardless of the cause of injury, and that other reactions were much more frequent than periderm formation.

Discussion

Although series I–III were of no great value in providing answers to the main questions under investigation, they were of interest since they apparently showed that *Thielavia basicola* may lose its virulence after long culturing on artificial media. This is not in accordance with the experience of JOHNSON and HARTMAN (**15**), who found no evidence in the literature or in their experiments of variation in virulence due to the age of the culture or to differences in



FIGS. 33–37.—Fig. 33, infected stem base showing cell divisions between fungus and periphery of stem; infected cells and those adjacent show metacutinization of walls (*chl*, chlamydospores of *Thielavia*); Havana 38, 28°–30° C., −N. Fig. 34, evidences of activity in pericycle adjacent to infected cells of cortex; walls of cortical cells and outer cells of pericycle, metacutinized (*ap*, activity in pericycle); Ordinary White Burley, 18°–20° C., +N. Fig. 35, infected root showing granulation of protoplasm in cells beneath heavily infected area (*end*, endoconidium; *gp*, granular protoplasm). No evidence of periderm formation around fungus lesion; Ordinary White Burley, 28°–30° C.,+N. Fig. 36, same stem base shown in fig. 34. No evidence that activity in pericycle has formed barrier of periderm cells in advance of fungus. Walls of infected and adjacent cells metacutinized, and granular gumlike material seen in some cells. Fig. 37, healthy portion of root shown in fig. 35; protoplasm of cells not granular.

strain, and that *Thielavia* is apparently stable in its pathogenicity. PETERS (21) and SATTLER (23) on the other hand both report loss of virulence after long cultivation on artificial media, two to three years according to PETERS. Both also report evidences of different biologic races of *Thielavia*.

The relation of soil temperature to disease has been fully discussed by JONES, JOHNSON, and DICKSON (16) and others. The effects of temperature on the resistance and susceptibility of tobacco to *Thielavia* have been well established by field observations and experiments, although the mechanism by which high temperature increases resistance is not understood.

Work on the relation of nutrition to resistance and susceptibility shows that, owing to the many factors involved in the nutrition of a plant, it is difficult to make generalized statements. FISCHER and GAÜMANN (13) state that in general, high nitrogen nutrition increases susceptibility, while high phosphorus, potassium, and calcium lessen it. They state that it is necessary to have extremes of high or low nutrition with these elements for the changes in resistance to be evident. Furthermore, the effects of these elements are not always the same. In certain diseases, high nitrogen may increase resistance and high phosphorus decrease it.

SCHAFFNIT and VOLK (24), working on the relation of various individual nutrients to resistance of both herbaceous and woody plants, found that plants with a decided lack of nitrogen were only slightly susceptible while those with a decided excess were especially susceptible. This was true wherever susceptibility and resistance were independent of the age of the host. In plants in which age affects resistance, this relationship to nitrogen may be reversed in the early stages of growth, while in the older stages the +N plants are again more susceptible.

LINK and WILCOX (20) report that succulent shoots of Stayman apple trees, such as formed under high nitrogen conditions, are susceptible to *Erwinia amylovora*, while the −N shoots are only slightly disposed toward the organism.

In the case of stem rust of wheat, a high amount of nitrogen is known to increase development of the rust. JOHNSON and JOHNSON (14) studied the nitrogen content of mature and immature parts of

the wheat plant in relation to resistance to the rust. They found that the mature parts had a higher nitrogen content than the immature parts, and at the same time were more resistant than the immature. They concluded that resistance cannot be due to the actual increase in nitrogen content, as believed by GASNER and FRANKE, but that in the older tissues other factors enter in, such as the increased resistance offered by the more mature cells or the development of some substance having an inhibiting effect on the fungus. They believe that resistance to rust cannot be explained on a directly nutritional basis.

Reports on the effects of nutrition on resistance to root fungi are not numerous, and the conclusions are somewhat divergent. SATTLER (**23**) found that beans were more susceptible to *Thielavia* under conditions in which nitrogen was either completely lacking or greatly in excess.

COOK (**11**) found that both resistant and susceptible tomato plants showed a high frequency of infection with *Fusarium lycopersici* under −N conditions, although the symptoms of wilt were infrequent in −N plants. Seedlings of a resistant variety were infected under both +N and −N conditions, showing symptoms similar to those of susceptible varieties.

REED and FRÉMONT (**22**) found that citrus roots growing in soils which had been without fertilizer for seven years offered little resistance to the invasion of mycorrhizal fungi and were unable to digest intracellular mycelium. Roots in soils having had annual cover crops and application of stable manure offered a definite resistance to the fungus.

THOMAS (**25**) states that trees infected with *Armillaria mellea* develop an antagonism to the fungus only when the plant is in an active healthy state, a condition which implies at least no lack of nitrogen.

The tobacco plants examined in the present study showed no anatomical modifications in response to the presence of *Thielavia* which could be correlated with resistance. No such periderm layers as described by CONANT were found; and while evidences of cell divisions around some infected areas were noted, these were not found consistently enough, either in a given diseased root or under

a given set of conditions, to be considered a factor in resistance. One of these regions showing such activity in the pericycle occurred in susceptible White Burley at the low temperature, the temperature condition under which the plant was the most susceptible (fig. 34). Other parts of the same crown showed that the fungus had not been stopped by a periderm-like barrier, but had penetrated the phloem almost to the xylem (fig. 36). This same irregularity of cell division in response to the presence of the fungus was seen in the other varieties used in this experiment.

Periderm formation has received much attention as a possible factor in determining the ability of a plant both to resist initial invasion and to prevent further penetration after invasion. The work of ARTSCHWAGER and STARRETT (4) on sweet potato and gladiolus shows the importance of the ability of the root to heal rapidly the wounds made in harvesting. Unhealed wounds are portals of entry for pathogenic organisms during storage. Under conditions of high temperature and relative humidity, when periderm is formed most rapidly, rotting in storage is much decreased. According to APPEL (2) the cork layer must be thick, for many fungi are able to penetrate thin cork layers.

As to the effectiveness of a corky layer in preventing continued penetration after initial invasion, there is a variety of findings. BROOKS (8) states that with certain varieties of plants, resistance to some fungus diseases is related to the ability of the host to form cork barriers readily in response to attempted invasion. This cork may apparently completely check the advance of the fungus, so that its harmful effect on the plant as a whole is decreased. In the case of rough bark of aspen, caused by *Macrophoma tumefaciens*, as described by KAUFERT (17), the fungus penetrates the periderm. The host forms a new periderm in response to the wounding; the fungus penetrates this, and the alternate penetration and formation of new layers continue until there is a very thick layer of cork. This slow progress of the fungus restricts the organism to the outer cortex although it may be present there for years.

THOMAS (25) found that the wound made by the invasion of *Armillaria mellea* in resistant hosts is walled off by a periderm, but

he doubts the significance of the cork layer as a factor in resistance, since the fungus easily breaks through such layers.

Much of the literature indicates that where cork formation is observed, it is not considered to be the only factor operative in resistance but functions in conjunction with other factors. APPEL (2) found in black leg of potato that the rapidity with which the potato is able to form cork in response to the wound is related to the ability to resist further invasion. But he also states that the efficiency of the cork layer is aided by conditions that diminish the growth of the bacteria, giving the wound time to heal before the bacteria can penetrate. In the case of fungus diseases such a newly formed cork is not effective, he thinks, as fungi are able to penetrate it.

LINK and WILCOX (20) found that a periderm walled off lesions made by *Erwinia amylovora* in apple shoots. But they also noticed that there was usually a band of unaffected cells between the bacteria and the periderm. The bacteria were evidently stopped first and the periderm was formed later as a regenerative reaction to the wound, rather than in advance as a defense mechanism.

TISDALE'S work on flax is often quoted as describing a situation in which the flax wilt fungus is prevented from invading the vessels in resistant varieties by the formation of a corky barrier between the fungus and the vessels. TISDALE in fact pointed out that the formation of a corky barrier was but one of a number of cellular changes, and that a substance was formed by the host cells which was possibly harmful to the fungus. He surmised that failure of the fungus to penetrate the periderm layer may be conditioned only after the fungus has been weakened by some activity on the part of the protoplasm of the host. Thus resistance resulted from a combination of the weakening of the parasite and the formation of a corky barrier.

The failure of BOYLE (5) to find in flax roots a corky layer such as described by TISDALE may be additional evidence that periderm formation is not the primary factor in resistance in flax roots.

BRAMBLE (6) was uncertain of the complete effectiveness of a wound periderm in preventing the spread of *Endothia parasitica* in chestnut. He thought, however, that it might delay or stop the ad-

vance of the fungus under conditions where the fungus grows slowly because of some other inhibiting factor. BRAMBLE concluded that wound periderm formation should at least be considered among the factors contributing to resistance to *Endothia*.

The metacutinization and thickening of the cell walls in infected areas might offer some mechanical resistance to invading fungi. But there was no evidence in the tobacco roots of this experiment that the fungus is restricted by these features, nor was there any correlation between their occurrence and resistance. From the comparable reactions in mechanically injured roots it is concluded that these are the usual wound responses.

Both TISDALE and BOYLE described metacutinization in flax, but from microchemical tests BOYLE concluded that the substance deposited in the cell walls is lignin-like. He found that this metacutinization is of the same type as that found in reactions to mechanical injuries, and he thought that the deposition of lignified material on the cell wall could not be considered a primary factor in resistance. BRAMBLE described similar reactions in chestnut, and concluded that lignification of cells was not of great importance in resistance to further invasion, since these cells also become infected.

BOYLE, however, found that a greater stability of the cortical cell walls was correlated with resistance. This stability was not evident anatomically but was detected by testing the resistance of the walls to hydrolysis with sulphuric acid and determining the amount of non-hydrolyzable materials in the walls.

Callus formation occurring as a result of severe injury is primarily a regenerative rather than a directly defensive reaction. Its occurrence in regions infected severely enough to result in the destruction of areas of cells suggests that the fungus has been weakened or stopped in its progress, giving the cells which have not been destroyed the opportunity to divide and begin regeneration. The newly formed callus is apparently not infected by the fungus remaining near the new tissue. This may be a further indication that the aggressivity of the fungus has been decreased, either because the organism has been weakened by some substance formed in the cells in response to the fungus, as suggested by TISDALE, or because the nutritive conditions are no longer favorable to the fungus. On the

other hand, the lack of infection in the new callus may suggest some chemical characteristic of this tissue as yet not understood. Anatomically callus does not offer a mechanical barrier to invading fungi, because of its thin walled cells.

Since no anatomical modifications were found in the tobacco plants of this experiment which could explain resistance and susceptibility to *Thielavia*, it seems probable that the mechanisms of resistance are chemical. TISDALE's suggestion of a fungus-weakening substance, the description by THOMAS of the development in roots of an antagonism toward *Armillaria mellea*, the mention by others of the formation of inhibiting substances, and the work on acquired physiological immunity in plants summarized by CHESTER (9), all show the trend of thought in the direction of a chemical basis for resistance. More specifically, the reports of ARNAUDI (3) that he was able to increase the resistance of tobacco plants to *Thielavia* by treating the plants with vaccines is suggestive of an approach to the understanding of the problem of resistance in the case of black root rot of tobacco.

Summary

1. Plus and minus nitrogen tobacco plants, of the five varieties used in this experiment, showed no differences in resistance and susceptibility to *Thielavia basicola* at any given temperature. Changes in nutrition do not change the order of resistance.

2. These experiments confirm the evidence of other investigators that soil temperature is the most important environmental condition determining the expression of resistance and susceptibility of tobacco to the fungus. The breeding of resistant varieties is the most important practical method of control of black root rot.

3. Periderm formation in control plants at different temperatures and under +N and −N conditions could not be correlated with resistance.

4. No evidence was found of the formation of periderm in advance of the fungus or around lesions made by the fungus.

5. Response to the wounds made by the fungus was similar to the reaction to mechanical wounds.

6. Callus formation in roots and stems in response to injury by the fungus appears to be regenerative rather than defensive.

7. Resistance to *Thielavia* under certain environmental conditions is not determined primarily by anatomical modifications in the root or crown of the plant.

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NITROGEN AND CARBOHYDRATE METABOLISM OF KIDNEY BEAN CUTTINGS AS AFFECTED BY TREATMENT WITH INDOLEACETIC ACID

NEIL W. STUART

(WITH FOUR FIGURES)

Introduction

It is well known that a large group of chemical growth substances are capable of inducing root formation on cuttings of many species, being particularly effective with softwood and herbaceous cuttings. Little is known, however, as to the nature of the chemical changes brought about within the cutting as the result of treatment with any of these growth substances.

STUART and MARTH (5) found that cuttings of *Ilex opaca* treated with indolebutyric acid accumulated more sugars in their stems prior to root emergence than did similar untreated cuttings. COOPER (2) had previously referred to this mobilizing action of growth substances in woody cuttings. He postulated that the action of indoleacetic acid in root formation is primarily the mobilization of naturally occurring root forming substances.

Regarding the effect of growth substances on the chemical changes of intact plants, rather than of cuttings, MITCHELL and MARTIN (4) applied 3 per cent indoleacetic acid-lanolin mixture to the first internode of etiolated kidney bean seedlings, and found that this treatment tended to restrict the withdrawal of materials from the cotyledon and their deposition in the treated portions and parts of the plant above the point of treatment. On the other hand, hypocotyls of treated plants gained more in dry weight following application of the acid than did similar parts of control plants. BORTHWICK, HAMNER, and PARKER (1) reported that in tomato stems treated with indoleacetic acid-lanolin mixture, proteins increased and starch decreased in those areas where cell divisions became most abundant. Their observations were made by means of microchemical tests.

The purpose of the present study was to determine the nature and

magnitude of the changes in dry substance as well as the distribution of nitrogen and carbohydrates in cuttings treated with indoleacetic acid. Cuttings of kidney bean seedlings were found to be very suitable for this study, being uniform and highly reactive to growth substances.

Materials and methods

Five crops of beans (*Phaseolus vulgaris*, Calapproved selection) were grown in the greenhouse during the spring and summer of 1938. The beans were grown in 300 5-inch pots each containing five beans planted at a uniform depth and position in a mixture of equal parts by volume of soil and peat. The beans were watered with tap water and received one application of a complete nutrient solution containing also the known essential minor elements.

Cuttings were made on the ninth day after planting, when the second internode was about 1 cm. in length (fig. 1). The hypocotyls were severed just above the surface of the soil. Except as otherwise noted, the cotyledons and the top of the plant above the heart-shaped leaves were removed when the cuttings were made. The cuttings, carefully selected for uniformity, were treated by standing them in bundles of twenty-five in 100 cc. beakers containing 50 cc. of 0.01 per cent indoleacetic acid or tap water for 4 hours. In preliminary experiments this treatment produced the maximum response. After treatment the cuttings were rinsed in tap water and set in media of washed sand contained in a glass inclosed propagating frame within the greenhouse. The frame was shaded so that the light intensity at the surface of the leaves was from 600 to 1000 foot candles. The temperature in the greenhouse ranged from 70° to 100° F. Each sample, consisting of 100 cuttings, was removed from the sand at 9 A.M., washed, and separated into upper and lower hypocotyls by division at the middle, first internode, petioles, and blades. These fractions were dried for 20 hours at 80° C. in a forced draft oven.

The sugar content of the cuttings was determined as previously described (**5**). Starch was digested with fresh saliva, followed by acid hydrolysis. Total nitrogen was determined by the Official Gunning method. No nitrates were present in the samples.

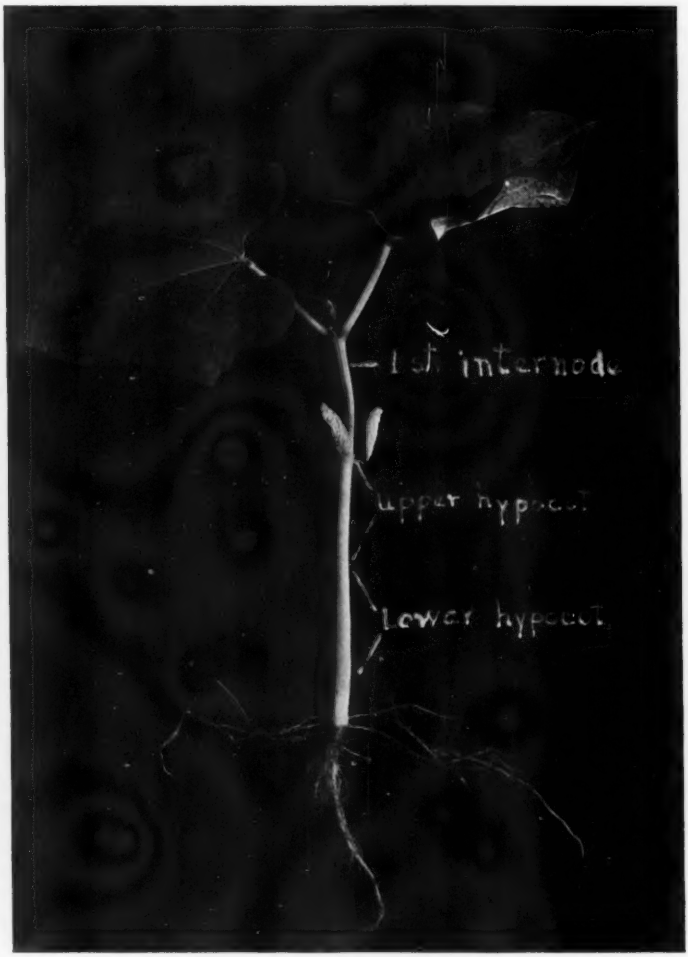

FIG. 1.—Stage of kidney bean seedling when cuttings were made

Experimental results

Influence of indoleacetic acid on growth of bean cuttings

Untreated cuttings produce a few roots and the base of the hypocotyl enlarges slightly by about 100 hours. During this period the second internode elongates and the first trifoliate leaves unfold. Similar cuttings treated 4 hours with 0.01 per cent indoleacetic acid produce four dense rows of roots on the hypocotyl, which enlarges greatly, particularly the lower portion. Roots are produced on treated and untreated cuttings at about the same time. Meanwhile the growth of the second internode is suppressed for several days, but gradually is resumed and the trifoliate leaves are unfolded (fig. 2). The response to treatment depends upon the concentration of the solution as well as upon the time of exposure. The responses obtained when cuttings are treated with 0.01 per cent indoleacetic acid for periods ranging from 1 minute to 4 hours are shown in figures 3 and 4. Lengthening the period of exposure increases the number of roots and size of hypocotyl (fig. 3), while the growth of the second internode is progressively depressed (fig. 4). Exactly the same effect is obtained if cuttings are treated for a uniform length of time with solutions of different concentrations of indoleacetic acid. The cuttings shown in figures 3 and 4 were treated with solutions ranging in concentration from 0.0001 to 0.01 per cent. Quantitative dry weight determinations were made, confirming the conclusions reached with respect to the visible growth responses.

Influence of indoleacetic acid on distribution of dry weight

The cuttings were grown under conditions of low light intensity and high temperature. As a result, there was little net increase in weight during the period in the propagation frame (tables 1 and 2). The untreated cuttings increased slightly more in dry weight than the treated ones.

There were, however, striking changes in the distribution of materials within the cuttings. The treated hypocotyls in a period of 120 hours nearly doubled in dry weight while the untreated hypocotyls increased only 20 per cent. On the other hand, the first inter-

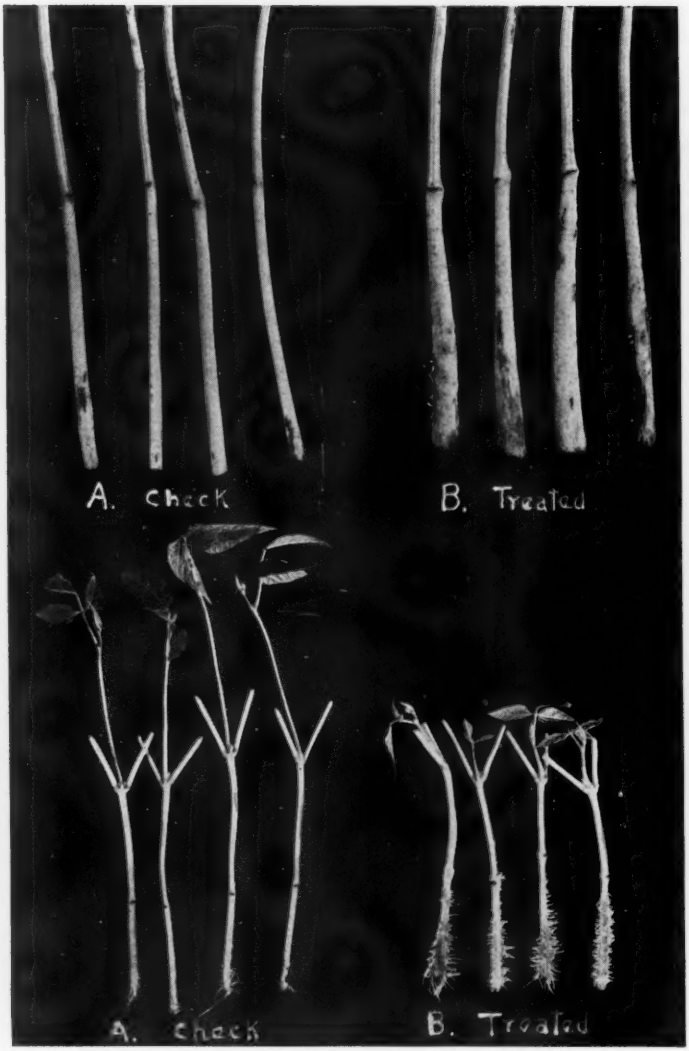

FIG. 2.—Upper: *A*, control cuttings 96 hours after making them; *B*, treated 4 hours with 0.01 per cent indoleacetic acid, 96 hours after making cuttings. Note increase in size of hypocotyls. Lower: same as upper, showing whole cuttings (except primary leaves) 120 hours after making them.

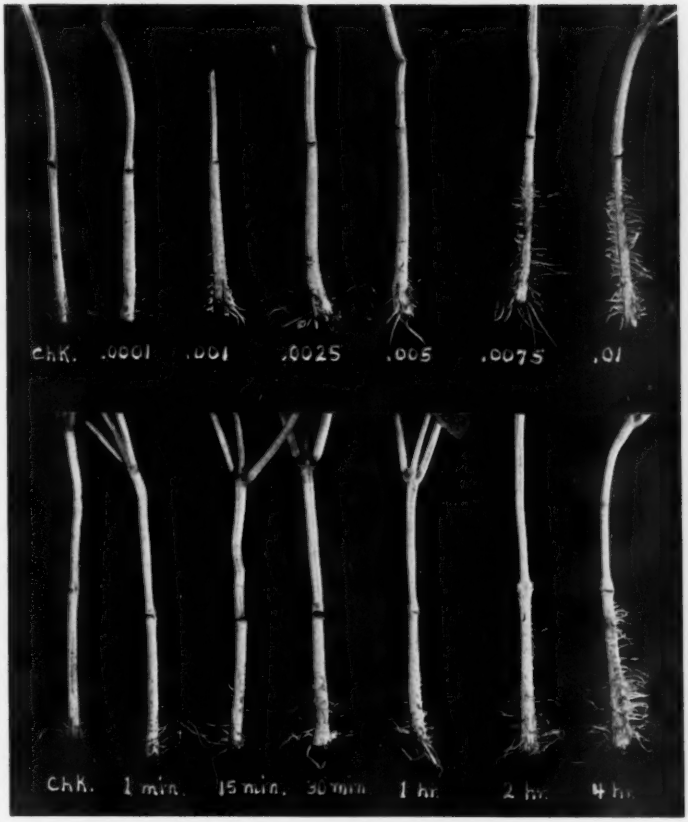

FIG. 3.—Upper: response of cuttings treated 4 hours with increasing concentrations of indoleacetic acid. Left to right, 116 hours after treatment. Note progressive increase in number of roots and size of hypocotyl. Lower: response when treated with 0.01 per cent indoleacetic acid for increasing lengths of time, 116 hours after treatment.

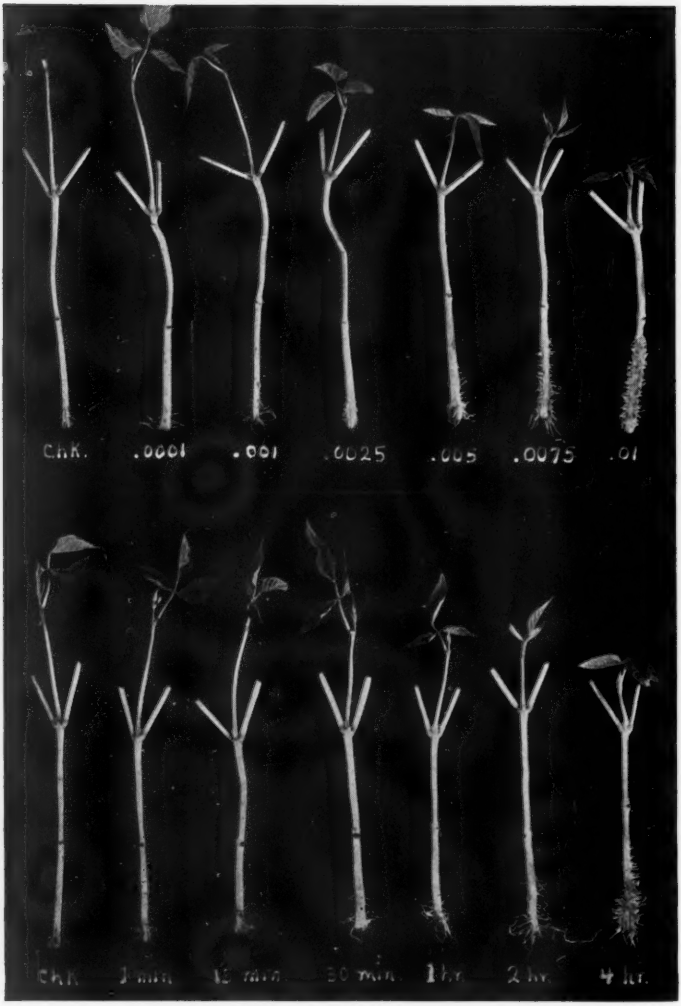

FIG. 4.—Upper: response of cuttings treated 4 hours with increasing concentrations of indoleacetic acid, 116 hours after treatment. Note progressive suppression of top growth. Lower: response when treated with 0.01 per cent indoleacetic acid for increasing lengths of time, 116 hours after treatment.

node and petioles from the control cuttings grew much more than those of the treated ones. In both treated and untreated cuttings the dry weight of the leaf blades decreased but the decrease was greater in the leaves from the treated cuttings. The results reported in table 1 are from cuttings in which the leaf area was restricted to the primary leaves and the cotyledons removed when the cuttings

TABLE 1

KIDNEY BEAN CUTTINGS TREATED JUNE 27 WITH 0.01 PER CENT INDOLEACETIC ACID FOR 4 HOURS IN COMPARISON WITH WATER

CUTTINGS SET IN SAND AFTER TREATMENT

Hours after treatment	Hypocotyls						First internode		Petioles		Blades		Total	
	Lower		Upper		Total									
	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.
	Dry weight (gm.) of 100 cuttings													
0	3.15	.....	2.31	.....	5.46	.....	2.72	.....	2.07	.....	16.4	.....	26.7	....
24	3.29	3.35	2.47	2.50	5.76	5.85	2.96	2.75	2.17	2.08	15.4	14.9	26.3	25.6
48	3.32	3.74	2.40	3.03	5.72	6.77	3.03	2.72	2.23	2.08	14.8	14.3	25.8	25.9
72	3.21	4.35	2.75	3.16	5.96	7.51	3.29	2.87	2.57	2.08	14.7	13.8	26.5	26.3
96	3.61	5.53	2.87	3.25	6.48	8.78	3.70	2.95	2.76	2.07	14.6	13.1	27.5	26.9
120	3.66	6.30	2.94	3.17	6.60	9.47	3.83	2.84	2.82	2.01	14.2	12.5	27.5	26.8
	Starch (mg.) in 100 cuttings													
0	37.8	.....	24.9	.....	62.7	.....	None	.....	Trace	.....	None	.....	62.7	.....
24	28.6	<10	<15	Trace	28.6	Trace	Trace	None	Trace	None	"	None	28.6	Trace
48	34.5	21.3	25.2	<10	59.7	21.3	56.7	None	43.3	None	"	"	159.7	21.3
72	66.8	36.1	72.9	32.2	139.7	68.3	90.1	Trace	52.9	Trace	"	"	282.7	68.3
96	48.7	38.2	91.3	22.1	140.0	60.3	137.3	None	62.1	Trace	"	"	339.4	60.3
120	50.9	30.9	85.8	<10	136.7	30.9	121.8	None	71.9	None	"	"	330.4	30.9

were made. Substantially the same results were obtained when the cotyledons were not removed and the second internode allowed to grow (table 2). It is apparent from these tables that the treatment exerted a powerful force in altering the growth response and distribution of total solids within the cuttings.

Influence of indoleacetic acid on the absorption of water

The great increase in dry weight of treated over untreated hypocotyls shown in table 2 was accompanied, as might be expected, by a very great increase in the amount of water. At the time of making

TABLE 2

KIDNEY BEAN CUTTINGS TREATED MAY 23 WITH 0.01 PER CENT INDOLEACETIC ACID FOR 4 HOURS IN COMPARISON WITH WATER. CUTTINGS SET IN SAND AFTER TREATMENT. COTYLEDONS AND SECOND INTERNODES NOT REMOVED

Hours after treatment	Hypocotyls						First internode		Petioles		Blades		Cotyledons		Total	
	Lower		Upper		Total											
	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.
	Dry weight (gm.) per 100 cuttings															
0	2.52	.....	2.12	.....	4.64	.....	1.40	.....	1.83	.....	16.8	.....	6.23	.....	30.9	.....
12	2.62	2.82	2.17	2.45	4.79	5.27	1.87	1.95	1.83	2.13	16.4	17.4	5.30	5.17	30.2	31.9
24	2.87	3.37	2.20	2.72	5.07	6.09	1.83	2.32	1.90	2.13	15.3	15.9	5.03	4.22	29.1	30.7
48	2.98	3.73	3.08	3.48	6.06	7.21	2.57	2.35	2.77	2.08	16.4	14.8	3.93	3.30	31.7	29.7
96	3.82	5.53	3.52	4.40	7.34	9.93	3.28	2.73	2.67	2.07	15.4	14.3	3.20	2.88	31.9	31.9
144	4.43	8.03	3.63	4.63	8.06	12.66	4.05	2.77	3.15	2.10	16.0	14.6	2.42	0.95	33.7	33.1
	Total nitrogen (mg. in 100 cuttings															
0	155	.....	125	.....	280	.....	65	.....	92	.....	1067	.....	205	.....	1709	.....
12	150	161	122	135	272	296	81	85	84	89	1018	1032	147	144	1600	1646
24	156	198	123	157	279	355	84	101	86	94	971	1013	146	104	1566	1667
48	147	222	150	213	297	435	102	119	113	88	956	886	98	66	1566	1593
96	172	330	161	288	333	618	137	165	98	83	873	752	55	42	1496	1660
144	174	448	149	306	323	754	161	178	121	85	861	629	50	12	1516*	1658

* Check cuttings slightly lower, owing to greater growth of second internode (discarded).

the cuttings the fresh weight of 100 hypocotyls was 62.9 gm., representing 58.2 gm. of water (92.5 per cent). Six days later the control hypocotyls weighed 72.7 gm., whereas the treated ones had increased to 155.0 gm. On a percentage basis, however, the difference in moisture content was not great, the controls having 88.8 per cent and the treated ones 91.8 per cent. In the other portions of the cuttings the percentage of moisture was always lower in the controls. However, owing to the slightly greater growth of the portions other than the hypocotyls in the controls, their total content of water was slightly greater than in the corresponding portions of the treated cuttings. Considering the whole cutting, treatment with indoleacetic acid caused an increased absorption of water over that taken up by the controls.

The total pectin content of the hypocotyls was determined in an effort to explain the greater water holding capacity due to treatment with the acid. These data show that the initial control, final control, and treated hypocotyls contained 136, 235, and 495 mg. respectively of unpurified calcium pectate.

Influence of indoleacetic acid on distribution of carbohydrates

The first effect of treatment was to cause a rapid mobilization of sugars in the hypocotyl (table 3). At the end of 48 hours the treated cuttings contained 6.6 mg. of sugar per hypocotyl while the controls had but 3.8 mg. More than half of this increase was due to sucrose. From then until the roots had emerged, at the end of 120 hours, the treated hypocotyls steadily decreased in sugars until they contained less than the initial controls. Meanwhile the control hypocotyls slowly increased in sugars.

Sugars also steadily accumulated in the first internode of the control cuttings. Exactly the reverse response was noted in the first internode of the treated cuttings, where at the end of 120 hours not a trace of sugar remained. To a slightly less extent the same response was noted in the petioles. In the leaf blades, too, the content of sugars was always less in those from the treated cuttings. This lower sugar content may have been due partly to some depressing action of indoleacetic acid on photosynthesis. However, the loss in

dry weight of the blades, coupled with the increase in weight of the hypocotyls, makes it appear certain that sugars as well as other materials were rapidly transported out of the leaf blades. Whether this is due directly to the stimulus of the acid or is caused by the

TABLE 3

KIDNEY BEAN CUTTINGS TREATED JUNE 27 WITH 0.01 PER CENT INDOLE-ACETIC ACID FOR 4 HOURS IN COMPARISON WITH WATER

CUTTINGS SET IN SAND AFTER TREATMENT

Hours after treatment	Hypocotyls						First internode		Petioles		Blades		Total	
	Lower		Upper		Total									
	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.	Check	Iac.
	Direct reducing sugars (mg.) in 100 cuttings													
0	151	.....	119	.....	270	.....	186	.....	128	.....	466	.....	1050	.....
24	117	194	111	159	228	353	208	187	136	117	178	166	750	823
48	149	209	126	187	275	396	258	162	183	121	271	180	987	859
72	141	179	147	174	288	353	245	132	209	109	185	198	927	792
96	161	122	131	108	292	230	231	59	209	68	227	125	959	482
120	147	120	148	68	295	188	241	None	123	29	169	37	828	254
	Sucrose (mg.) in 100 cuttings													
0	22.4	.....	20.3	.....	42.7	.....	54.4	.....	20.1	.....	141	.....	258	.....
24	13.8	27.5	11.9	27.0	25.7	54.5	38.2	22.3	14.3	8.3	121	121	199	206
48	40.2	138.8	47.8	96.7	88.0	235.5	72.4	58.2	36.8	20.4	127	156	324	470
72	55.9	111.4	42.4	76.2	98.3	187.6	65.8	65.4	33.4	16.8	111	46	309	316
96	54.2	84.6	51.7	39.0	105.9	123.6	85.8	26.0	35.1	15.3	114	99	341	264
120	58.6	53.6	48.2	15.2	106.8	68.8	63.2	None	42.9	13.7	102	96	315	179
	Total sugars (mg.) in 100 cuttings													
0	181	.....	147	.....	328	.....	244	.....	154	.....	638	.....	1364	.....
24	137	231	129	195	266	426	248	218	158	131	319	306	991	1081
48	197	364	182	296	379	660	344	230	230	148	420	360	1373	1398
72	206	301	198	261	404	562	324	207	253	132	278	260	1259	1161
96	225	218	192	149	417	367	331	90	255	88	362	239	1365	784
120	216	182	199	84	415	266	317	None	174	47	292	139	1198	452

greatly increased meristematic activity in the hypocotyl is not known.

It should be noted that there was a net loss of more than two-thirds of the sugar originally present and synthesized by the treated cuttings during the 120 hours in the propagating frame. The controls showed a net loss of only 12 per cent. The initial decrease in carbohydrates from both treated and control cuttings during the first 24 hours after treatment is probably due to interference with

photosynthesis while the cuttings were being made, treated, and set in the propagating frame. Respiration losses were probably greater, too, owing to wound stimulus caused by making the cutting.

Under the conditions of this study the carbohydrate metabolism was concerned principally with the sugars. Starch tests were made on all samples, and quantitative determinations made whenever a sufficient amount was present to enable estimation by the method used (table 1). The initial cuttings contained starch only in the hypocotyls. During the period in the propagating frame, additional starch was deposited in the control hypocotyls and still greater amounts laid down in the first internode and petioles. The treated cuttings never stored starch above the hypocotyl, however, and the original amount there decreased somewhat. The carbohydrate analyses demonstrate the remarkable power of the indoleacetic acid to direct the distribution and metabolism of carbohydrates.

Influence of indoleacetic acid on distribution of total nitrogen

The bean cuttings used in this study contained sufficient nitrogenous reserves, exclusive of the cotyledons, to account (if calculated as protein) for more than one-third of all their dry weight. The effect of treatment was to cause a prompt mobilization of nitrogen from the leaf blades to the hypocotyl (table 2). Within 12 hours after treating, an appreciable amount of nitrogen had been translocated to the hypocotyls. After 24 hours the nitrogen content of the treated hypocotyls had increased nearly 27 per cent, while the control hypocotyls showed no change in nitrogen content. At the end of 144 hours more than ten times as much nitrogen had moved into the treated hypocotyls as into those of the controls.

In the control cuttings the same directional shift of nitrogen from the leaves and cotyledons and accumulation in other parts is noted, but without the influence of the indoleacetic acid treatment this movement was comparatively sluggish. In the treated cuttings it is apparent that, even at the end of 144 hours, rapid withdrawal of nitrogen from the leaf blades and cotyledons and its accumulation in the hypocotyls was still taking place, despite the considerable movement already accomplished.

Discussion

The prompt movement of large amounts of nitrogen and carbohydrates to the treated hypocotyls was correlated with the emergence of many more roots than appeared on the control hypocotyls. The accumulation of these food materials doubtless favored root development and meristematic activity. However, other substances as well as sugars and nitrogen may have been translocated to the hypocotyls under the influence of the treatment. In this connection, COOPER (**2**) has presented convincing evidence that cutting off the bases of leafless woody cuttings a short time after treating them effectually removes something which is associated with root formation. COOPER refers to this substance as rhizocaline. It would be of interest to know whether appreciable amounts of the common food materials were mobilized in his cuttings and hence lost when their bases were removed.

The temporary suppression in growth of the second internode of bean cuttings was probably caused in part by the movement of carbohydrates and nitrogen away from the apical regions. The comparative subsequent growth of treated and control cuttings after rooting is now under investigation.

Various other plant responses to the application of indoleacetic acid, including such phenomena as bending, gall formation, and even induced parthenocarpy, can possibly be explained through the action of the acid in mobilizing various materials to the region of application. The results obtained in this study help to explain also the striking morphological and histological changes in bean plants treated with indoleacetic acid as reported by KRAUS and co-workers (**3**).

The mechanism by which the indoleacetic acid so profoundly affects the movement of food materials is unknown. THIMANN and SWEENY (**6**) reported that low concentrations of indoleacetic acid accelerated the rate of protoplasmic streaming. It is not known to what extent the treatment used in the present study may have affected the transport of food materials by streaming. Further investigation is necessary before it is known whether the mobilizing action of the growth substances is due to the new meristems which

arise or to the direct action of the growth substances themselves, or to some other reaction not yet recognized.

Summary

1. Cuttings of kidney bean seedlings were treated by immersing their bases in 0.01 per cent indoleacetic acid for 4 hours. They were then set in sand in a propagating frame for 120 hours. During this period, in comparison with control cuttings, the treatment brought about a directional shift of large amounts of nitrogen and carbohydrates from the leaves and cotyledons to other portions of the cuttings, principally to the treated hypocotyls.

2. Accompanying this mobilization of materials, the treated cuttings responded by rapid swelling of the hypocotyls, profuse root production, and a temporary suppression of top growth. Total dry weight of the treated cuttings at the end of the rooting period was slightly less than that of the controls.

3. The responses were proportional to the length of exposure and the concentration of the acid.

4. The possible importance of indoleacetic acid as a mobilizer of food material in various other growth responses is suggested.

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THE AMERICAN SUGAR MAPLES

I. PHYLOGENETIC RELATIONSHIPS, AS DEDUCED FROM A STUDY OF LEAF VARIATION

EDGAR ANDERSON AND LESLIE HUBRICHT

(WITH FIVE FIGURES)

Introduction

For a number of reasons it seemed probable that the sugar maples of eastern North America (*Acer saccharum* in the broad sense) would provide interesting material for a study of variation. In the first place, the relationships of the various elements within these maples are complex enough to be interesting (table 1), but simple enough to give some hope of eventual solution and exposition. In the second place, sugar maples usually grow in large, fairly conspicuous groves, and it is therefore possible to locate and study representative populations with a minimum of effort. Finally, and of particular importance, a considerable proportion of the characters used in specific delimitation is represented in the leaves, so that a study limited mainly to leaf variation might be expected to yield significant data. Most of these characters are resolvable into lengths or angles and are therefore easy to record objectively and to treat statistically.

Methods

Collections of leaves, one from each tree, were made in various large groves as opportunity offered. Since the general problem was more concerned with differences between trees than with differences on a single tree, much of this extraneous variation was removed by a careful selection of comparable material. Collections were not made until after midsummer, when leaves were fully mature. So far as possible, leaves were collected only from mature or nearly mature trees, from lower branches, never from fruiting branchlets, never from branches in deep shade, and never from terminal twigs. Most students realize the varying effects of such factors; the problem has

been given thorough statistical treatment by JENTYS-SZAFEROWA (4) in the case of *Betula* leaves.

Each collection was provided with a name, usually geographical. The leaves were pressed like ordinary herbarium specimens but were left unmounted. After they were completely dry, the name of the collection and the number of the leaf were written on the lower surface in pencil. Each leaf was then scored for the characters diagrammed in figure 1. The degree of pubescence was also scored, the following grades being recognized:

A. Axils of primary veins on lower surface of leaf—
   0. Glabrous or essentially so
   1. Leaf visible through pubescence
   2. Leaf not visible through pubescence.

B. General lower surface of leaf—
   0. Glabrous or essentially so
   1. Covered with scattered hairs; not interlacing
   2. Covered with interlacing hairs.

C. Petiole—
   0. Glabrous or essentially so
   1. Pubescent only at junction with leaf
   2. Pubescent entire length.

Observations

As soon as a few representative populations had been studied, the complexity of the relationships of the various elements within the sugar maples became apparent. In each grove studied there was conspicuous variation from tree to tree. The collections from New England presented the simplest picture. The outlines of ten leaves chosen at random from the collection made at Petersham, Worcester County, Massachusetts, are illustrated in figure 2. Although there is considerable difference from leaf to leaf the variation is centered about a single plexus, and any one of these leaves could confidently be identified as belonging to *Acer saccharum* in the narrow sense.

The collections from southern Michigan were slightly more complex. The variation in these groves centered about two plexi, one

of which was practically identical with the *A. saccharum* of New England; the other was the element which we shall refer to *A. saccharum* var. *nigrum*. When the groves from southern Michigan were examined as wholes, it was evident that the variation between these two plexi was practically discontinuous. For example, of the forty-eight trees examined in the Nesbit grove, near Schoolcraft, Kalamazoo County, Michigan, thirty-one were definitely *A. saccharum*, fif-

TABLE 1

COMPARISON OF THREE TAXONOMIC TREATMENTS OF NORTHERN SUGAR MAPLES. HORIZONTAL DOTTED LINES SEPARATE VARIETIES; UNBROKEN LINES SEPARATE SPECIES

GRAY'S MANUAL	TRELEASE	REHDER'S MANUAL
A. saccharum Marsh	A. saccharum Marsh	A. saccharum Marsh
		A. saccharum var. glaucum Pax.
	A. saccharum var. barbatum Michx.	A. saccharum var. schneckii Rehd.
		A. saccharum var. rugelii (Pax.) Rehd.
A. saccharum var. nigrum (Michx. f.) Britton	A. saccharum var. nigrum (Michx. f.) Britton	A. nigrum Michx. f.
		A nigrum var. palmerii Sarg.

teen belonged to *A. saccharum* var. *nigrum*, and two were apparently intermediate.

The problem was made more complex by the inclusion of collections from Missouri and southern Illinois. The results of these collections were not only difficult to harmonize with those from the north; they did not even agree with each other. At some localities it was possible to divide the collections into two plexi, one of which was apparently the same as *A. saccharum* of Michigan and Massachusetts, although it differed more from them than they did from each other. The second was apparently *A. saccharum* var. *nigrum*, although it presented certain anomalies. In other groves no such discontinuity was perceptible. At such localities there were some

trees very similar to those of *A. saccharum* in Michigan and New England; there were other trees which on the basis of a single specimen might have been identified with *A. saccharum* var. *nigrum;* but there were other types not seen in the north, and the whole collection

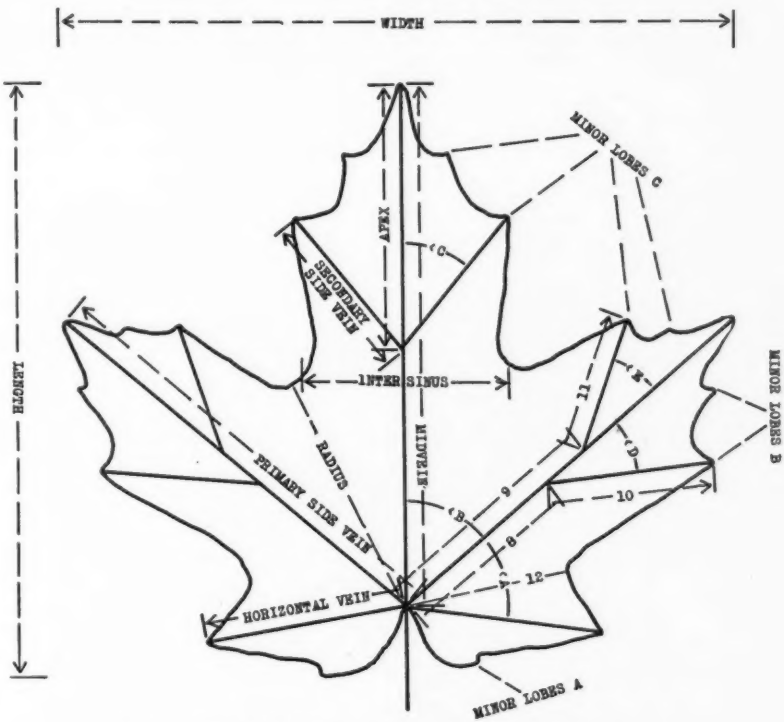

FIG. 1.—Maple leaf showing measurements and enumerations used

was so intricately related morphologically that no separate or even partially separate elements could be distinguished. The grove could be considered as a whole or as individual trees, but no other natural groupings of the material could be made. The outlines of ten leaves selected at random from the collection made at Fountain Gap, Monroe County, Illinois, will show something of the great variation encountered at such localities (fig. 2).

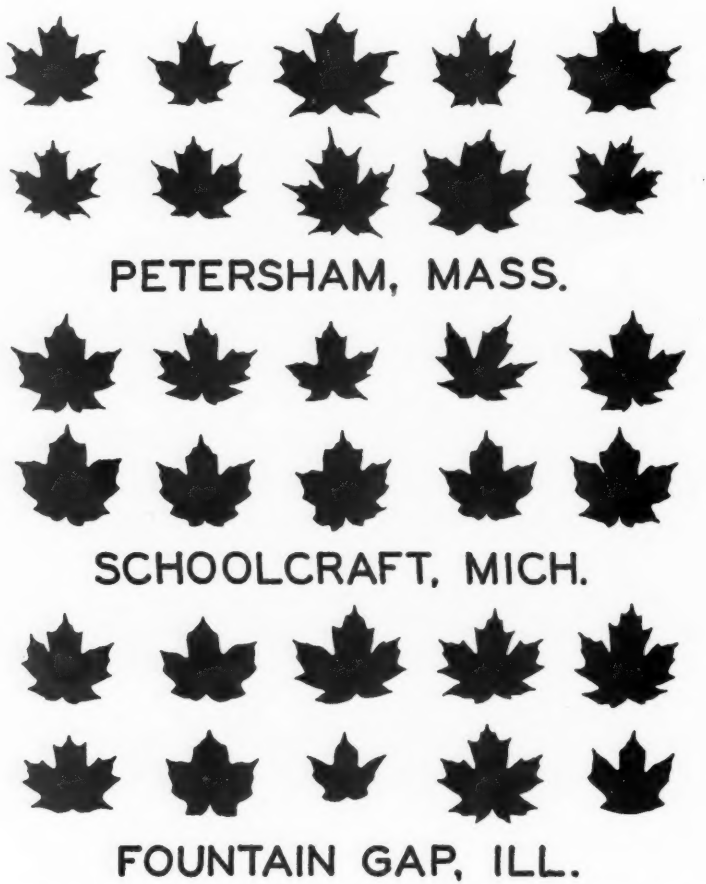

FIG. 2.—Variation between individuals. Outlines of representative leaves from ten trees at Petersham, Massachusetts (all *A. saccharum*), ten at the Nesbit grove near Schoolcraft, Michigan (five *A. saccharum* and five *A. saccharum* var. *nigrum*), and ten from Fountain Gap, Illinois (various species).

It is not the purpose of this paper to discuss these complexities. For the most part they have been adequately treated in such papers as that by TRELEASE (6). It is rather our purpose to show how a combined quantitative and qualitative study of the variation in these maples may lead to working hypotheses as to their relationships, and suggest new lines of attack.

There are two kinds of evidence in such problems, which are gleaned more reliably from the study of entire populations than from the study of single individuals. The first of these is an estimate of the discontinuity of variation. In studying sugar maples, for instance, a much more accurate reflection of the amount of intercrossing between *A. saccharum* and *A. saccharum* var. *nigrum* can be gained from a study of a population in which they both occur than from the usual herbarium material.

The second sort of data more reliably gained from whole populations is averages and their comparison. One of the chief advantages of quantitative characters is that they can be averaged and compared. On the other hand, averages of isolated characters are not particularly useful in discriminating between species, varieties, or races (1). Much more significant is the average of each character in relation to the average of all the other characters. An attempt was therefore made to turn the averages for each grove into a picture of what might be called the average leaf of that locality. The averages of the twenty-two measurements illustrated in figure 1 provided, when combined, the skeleton of such a leaf, with the main veins, sinuses, and apices determined, but without a margin. To complete the average leaf it was necessary to provide some kind of average margin. A working approximation was obtained in the following manner. Margins of sugar maple leaves differ in the number of serrations between apices, and in the curves of the actual margin. The margin was divided into six arcs on each side, marked by the apices and sinuses (fig. 1). For each of these arcs the average number of major serrations was calculated. To obtain an average value for the undulations of the margin within each arc, a tracing was made of that arc for each leaf. These tracings were then superimposed on the same base line and with the same point of origin (fig. 3). Knowing the average length and the average number of serrations, it was

comparatively easy from this composite tracing to draw, by inspection, a modal margin for each arc. These were then transferred to the average skeleton.

Average leaves prepared in this manner are shown in figure 4. Of particular significance are the averages for *A. saccharum* from Peter-

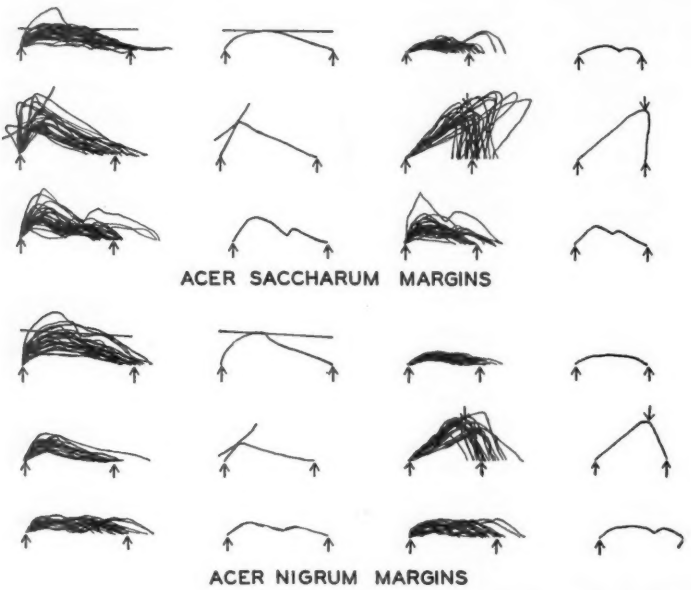

FIG. 3.—Graphical method for obtaining average of leaf margin; further explanation in text.

sham and from the Nesbit grove. Whatever their relationship nomenclatorially or phylogenetically, *A. saccharum* and *A. saccharum* var. *nigrum* are very similar. Although they are often found growing intermingled, flowering and fruiting at the same times (in the north at least), they remain distinct in spite of their obviously close relationship. Morphologically the total hiatus between them, in proportion to the variation within either element, is only a fraction of that usually found between closely related species. Unless the internal barrier is very powerful indeed, we should expect to find that *A. sac-*

charum growing in the same groves with *A. saccharum* var. *nigrum* would on the average be more *nigrum*-like than when it grows alone. The Petersham and Nesbit averages show no evidence of such an effect. They support the opinion of such students as L. H. BAILEY (2), who found that, in spite of their remarkable similarity and frequent juxtaposition, these two maples remain entirely distinct throughout much of their range.

To a cytogeneticist, these facts suggest a simple working hypothesis as to phylogenetic relationships. If the germplasm of *A. saccharum* var. *nigrum* were practically that of *A. saccharum* plus something else, and if this something else were added in such a way as to create an effective barrier to intercrossing (inversions, polyploidy, etc.), then we should expect just such a relationship. The something else would at the same time create a barrier to intercrossing and produce a slight but definite morphological hiatus between the original stock and its derivative variety. The preceding averages may be used to show what a maple would look like which could cause such an interaction with *A. saccharum*. On our hypothesis: *A. saccharum* var. *nigrum* = *A. saccharum* + x. The morphological relationships between these three elements should be as follows:[1]

$$\frac{A.\ saccharum}{A.\ saccharum \text{ var. } nigrum} = \frac{A.\ saccharum \text{ var. } nigrum}{x}$$

Since only one of these terms is unknown, it is a simple matter to solve the equation. If we determine statistically the appropriate lengths and angles for x and assemble them into a composite, we have the maple shown in figure 4.

We have thus obtained by statistical prediction the kind of a maple which by interaction with *A. saccharum* might produce such a maple as *A. saccharum* var. *nigrum*. The prediction is interesting since such a maple does exist and in eastern North America. It is in fact the *Acer rugelii* or *A. saccharum* var. *barbatum* of table 1. We have ourselves collected it frequently in the edge of the Ozarks; it is

[1] The actual value to be assigned to x would depend upon the relative proportions of x and *A. saccharum* in the germplasm of *A. saccharum* var. *nigrum*. Since as yet we have no information on that point, the calculations have been made on the simplest possible assumption: equal amounts of each. This would of course be the expected proportions for an amphidiploid relationship.

actually one of the complexities met with in such groves as that at Fountain Gap. Figure 5 shows how our predicted leaf is almost identical with one of the illustrations of *A. saccharum* var. *barbatum* published by TRELEASE in 1894.

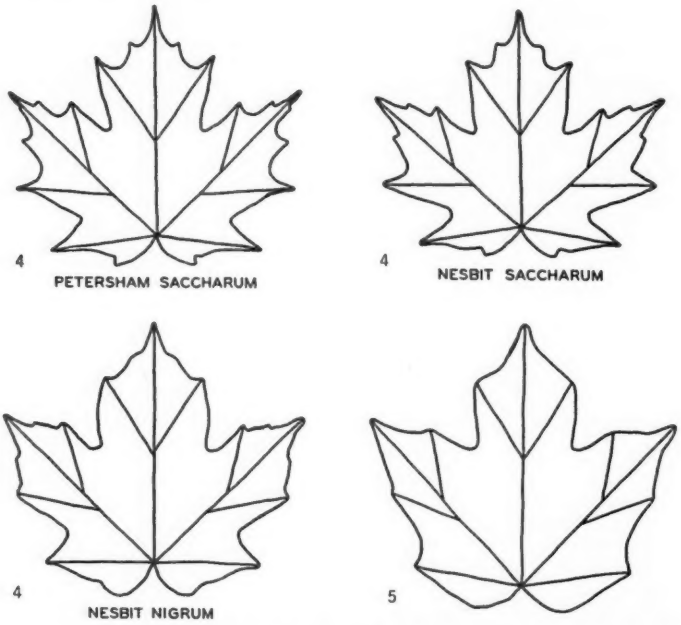

FIG. 4.—Average leaves for Petersham grove and for *A. saccharum* and *A. saccharum* var. *nigrum* in Nesbit grove. Lower righthand leaf (5) is a hypothetical one, determined by statistical prediction, for comparison with fig. 5, opposite page.

As a basis for further experiment, we therefore advance the hypothesis that *A. saccharum* and *A. saccharum* var. *rugelii* are the older elements in the North American sugar maples, and that *A. saccharum* var. *nigrum* has been derived from them, in some way as yet undetermined.

Summary

1. *Acer saccharum* (in the broad sense) is promising material for the study of variation, since: it presents an interesting but compara-

FIG. 5.—Reproduction of TRELEASE'S original drawning of *A. saccharum* var. *barbatum* (used by permission), for comparison with hypothetical leaf (fig. 5 on opposite page) determined by statistical prediction.

tively simple phylogenetic problem; it grows in large, easily located groves; most of the specific differences are confined to the leaves; many of these differences can be recorded as lines and angles and are then readily treated statistically.

2. Methods of collecting and measuring leaves are described.

3. Study of such collections shows: (a) The New England groves, while variable, centered around a single plexus,[2] *A. saccharum* (in the narrow sense). (b) The groves from southwestern Michigan centered around two almost discontinuous plexi, *A. saccharum* and *A. saccharum* var. *nigrum*. (c) The groves from southern Missouri and southern Illinois were sometimes like those from Michigan. Other groves from this region showed great variability but no distinguishable variation centers.

4. A method for reconstructing an average leaf from each population sample is described.

5. A study of such average leaves shows that *A. saccharum* var. *nigrum* had no effect on *A. saccharum*, even when they grew intermingled and flowered at the same time.

6. Possible phylogenetic relationships of *A. saccharum* and *A. saccharum* var. *nigrum* are discussed. On the hypothesis that *A. saccharum* var. *nigrum* represents *A. saccharum* modified by an unknown *Acer*, the characters of this unknown are determined by statistical prediction, and prove to be practically identical with *A. saccharum* var. *rugelii*.

7. It is therefore suggested, as a working hypothesis, that the older elements in the northeastern sugar maples are *A. saccharum* (in the narrow sense) and *A. saccharum* var. *rugelii;* and that *A. saccharum* var. *nigrum* represents some sort of interaction product between these two.

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[2] While *A. saccharum* var. *nigrum* does occur in certain parts of New England, it was not present in any of the groves studied.

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NOTES ON SOME PLANT REMAINS FROM THE CARBONIFEROUS OF ILLINOIS

FREDDA D. REED

(WITH NINETEEN FIGURES)

Introduction

The plant fragments here described were found in coal ball 236 of the Harrisburg collection, made at Harrisburg, Illinois by Dr. A. C. Noé, under the auspices of the Illinois State Geological Survey. The coal balls were found in coal seam no. 5, which is in the Alleghany group of the Upper Pennsylvanian. Although the mines have now been closed, thereby making it impossible to do further collecting in the locality, except from the mine dumps, there are still at hand a number of concretions awaiting examination.

The fragments consist of a stem tip surrounded by imbricated sheaths of leaf bases, of scattered leaves, and of some reproductive structures (sporangiophores with attached sporangia containing spores), none of which, in so far as I have been able to determine, are referable to any previously described forms from the Carboniferous of America. Scattered through the same coal ball were many other plant fragments, portions of leaves, petioles, stems, roots, sporangia, and synangia, all of them recognizable as belonging to well established Paleozoic forms. Since there are so many different genera, and since there is such diversity of both vegetative and reproductive structures so heterogeneously assembled and closely associated in the concretion, the mere fact of the presence of "unknown fragments" in the coal ball would be no basis for assuming relationship among them. Moreover, in the absence of any organic connection of the fragments under consideration, it is impossible to relate them definitely. Yet an examination of their structure reveals in each case certain features that indicate a Calamitean affinity for all of them. Therefore the stem, leaves, and sporangia are described separately and in order; not with any assurance that they belong to the same plant, but with the possibility that they may have been so associated

and with the hope that additional material and subsequent investigation will determine their relationship.

Observations

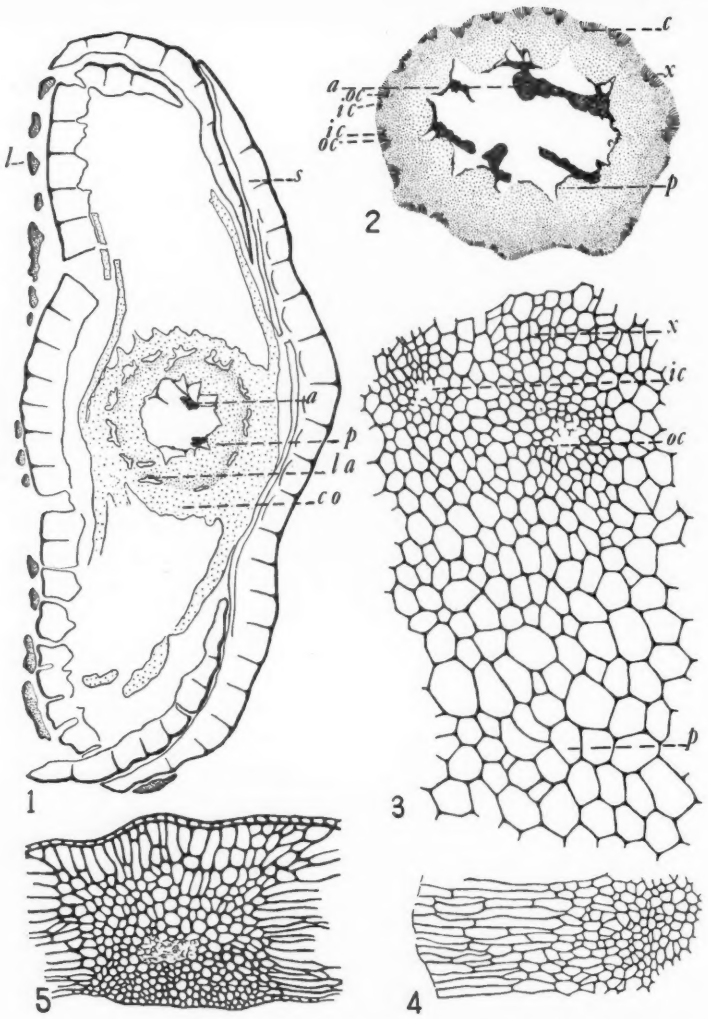
Stem

The stem fragment consists of a stem tip 3–4 cm. in length. In transverse section the pattern made by the stem and surrounding verticels of leaf bases is oval (fig. 1), but this shape may be the result of some external pressure before petrifaction occurred and it would otherwise be circular. The long diameter of the fragment, including the leaf bases as shown in figure 1, is 2.3 cm., and the short diameter is 1.5 cm. The diameter of the stem at the level of figure 1, which was taken near the base, is 7 mm., while toward the tip it narrows to a diameter of 3.5 mm.

The stem is hollow, with remnants of disintegrating tissue (figs. 1, 2*a*) extending into the fistular cavity just as sometimes may be found in modern *Equisetum* stems. The cavity is surrounded by a cylindrical mass of parenchymatous pith, which centripetally appears to be definitely organized into eleven wedge-shaped groups at the level of figure 2. The cells of the pith are relatively thick walled and vertically elongated (figs. 3, 6, 7); those toward the center are larger in transverse diameter, measuring about 70 μ. From the center outward they become increasingly smaller, until in the peripheral region they average about 20 μ (fig. 3).

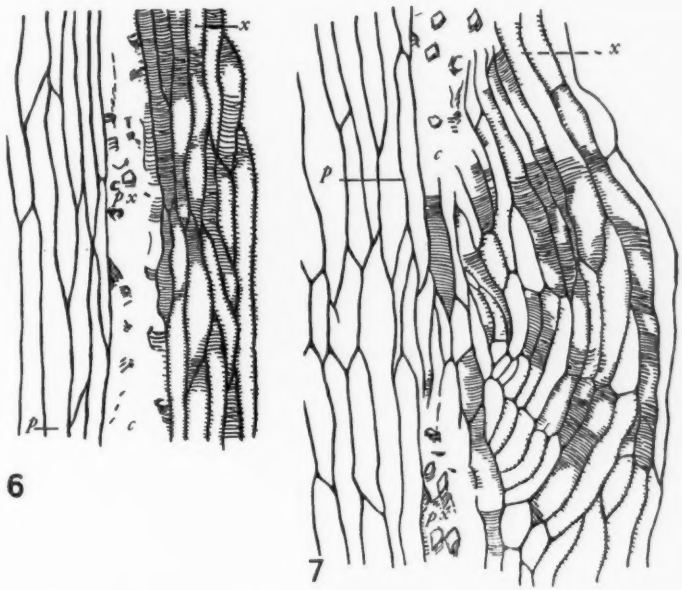
At the periphery of the pith zone are some canals from which the cells radiate centrifugally. The canals are possibly homologous with the carinal canals of *Calamites* and *Equisetum*. The cells radiating from them are interpreted as secondary wood. The canals are regularly disposed, four to each of the wedge-shaped groups; and of the four the two lateral (figs. 2, 3*oc*) are nearer the center than the two inner ones (figs. 2, 3*ic*).

In transverse section the peripheral cells, because of their size and the thickness of their walls, are indistinguishable from those of the adjacent xylem elements; limitation of the vascular strand is consequently obscure. A radial longitudinal section through one of the canals reveals the inner limit of the xylem, and indicates the method



FIGS. 1–5.—*Calamites multifolia*. Fig. 1, diagram of transverse section of stem and verticels of leaf sheaths: *l*, leaf; *s*, leaf sheath; *la*, lacunar area (probably region of phloem); *co*, cortex; *p*, pith; *a*, remnants of disintegrating pith tissue. × about 5. Fig. 2, transverse section of stele: *x*, secondary wood; *cc*, carinal canal; *ic*, *oc*, inner and outer canals of group of four; other lettering as in fig. 1. ×12. Fig. 3, detail of pith and wood of fig. 2. ×80. Fig. 4, detail of portion of cortex; inner cortex to right. ×35. Fig. 5, detail of segment of leaf sheath; vascular strand imperfectly preserved. ×35.

of formation of the canal as well. As shown in both figures 6 and 7, the canal is bordered on the inner side by pith and on the outer side by xylem; within are traces of disorganized xylem, the most complete disorganization being found on the side next to the pith. It is evident, therefore, that the canal is the result of disintegration of



FIGS. 6, 7.—*Calamites multifolia*. Fig. 6, radial longitudinal section through carinal canal; to the left is pith (*p*); in the canal are remnants of disorganizing protoxylem (*px*); to the right are scalariform tracheids of metaxylem and secondary xylem. ×107. Fig. 7, longitudinal section through nodal xylem, also showing disorganizing protoxylem. ×107.

xylem elements; also that the first elements, protoxylem, to break down were centripetal and that the bundle is endarch. The markings on the walls of the metaxylem and secondary xylem are scalariform to reticulate.

At the basal end of the fragment there is no structure preserved intact beyond the secondary wood (fig. 2). At a slightly higher level

the cortex is preserved yet partially separated from the woody zone by lacunar areas (fig. 1*la*). These lacunar areas provide the only clue to the former existence and position of the phloem, which was not preserved probably because of the soft texture of the cell walls.

The cells of the cortex show some variation; those of the inner region resemble the pith in size and structure, while those toward the exterior are radially elongated and radially arranged. The epidermis was not observed.

Toward the apical end of the fragment the tissues are progressively less mature, until at the apex there is no distinguishable organization into vascular bundles; instead the cells all appear to be parenchymatous and fairly uniform in size, with the cell walls appreciably thinner.

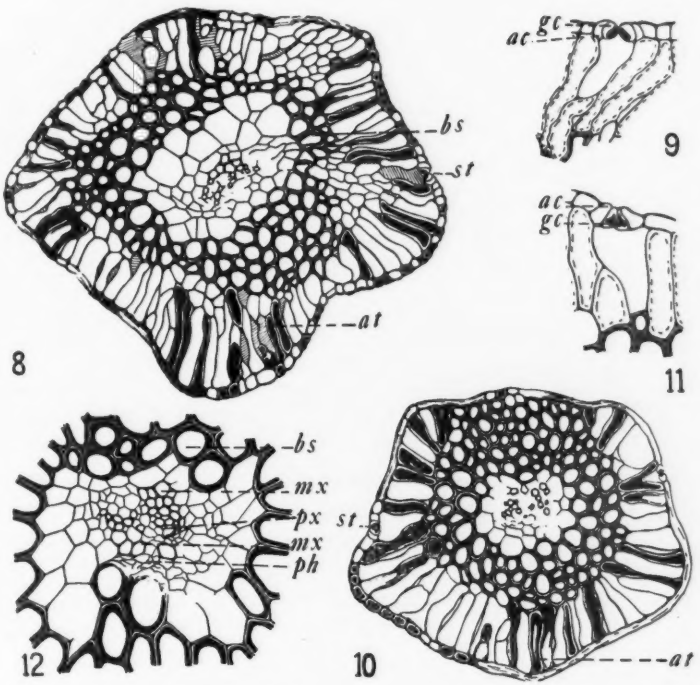
Beyond the cortex are the sheaths of leaf bases. These are somewhat crushed, are more or less completely preserved, and are partly separated by intruded fragments of other plants. Because of the incomplete preservation and fragmentation of the verticels of leaf sheaths, a detailed account of their number and structure is impossible. At the level of figure 2 there appear to be portions of two whorls, whereas at the tip there are four or five. When the sheath diverges from the stem it is composed of some forty coherent segments which at a higher level separate into as many discrete units, the leaves (fig. 1*l*). Each segment of the sheath is provided with a vascular strand.

LEAF

In almost every section of coal ball 236 there are portions of leaves variously cut which in their general features resemble those of *Calamites* (3). Some of the transverse sections are almost completely preserved, making it possible to work out the anatomical detail from that aspect. Unfortunately the longitudinal sections are not so satisfactory.

There is considerable range in the variability of the shape of the transverse sections of the leaves: most of them are roughly rectangular with a slight median ridge on the adaxial side and a convex abaxial surface (fig. 8); some of the leaves are more regularly rectangular than the one illustrated in figure 8; and an occasional one

may be found of the proportions shown in figure 10. The variation is in the degree of development of the several tissues rather than in any specific difference in organization, and the range of variability lies well within the limit found in different leaves of the same plant,



FIGS. 8–12.—*Asterophyllites* (*Calamocladus*) *multifolia*. Fig. 8, transverse section of leaf: *st*, stoma; *at*, assimilating tissue; *bs*, bundle sheath. ×80. Fig. 9, stoma from fig. 8: *gc*, guard cell; *ac*, accessory cell. ×160. Fig. 10, transverse section of leaf in which bundle sheath extends to epidermis on adaxial side. ×80. Fig. 11, detail of stoma of fig. 10. ×160. Fig. 12, vascular bundle of leaf: *px*, protoxylem; *mx*, metaxylem; *ph*, phloem. ×155.

or even within the limit that might be found in one leaf if it were possible to secure all the transverse sections from the base to the apex.

The leaves average about 0.8 mm. wide by 0.45 mm. thick. The

length was not determined, but one leaf followed through a distance of 5–6 mm. showed no diminution in width nor gave any indication of its extremities.

The epidermis is not irregularly cutinized, as has been found for some of the Calamitean leaves (3). In transverse section the epidermal cells are relatively small and are rectangular to square, many of them filled with dark brown contents. Only rarely are the stomata found, but their seeming sparseness can probably be attributed to the imperfection of preservation of the epidermis and to their very small size, which renders them obscure except under high magnification. Those observed were on the edges of the leaf (figs. 8, 10). The guard cells are on the same level or only slightly below the level of the other epidermal cells, are somewhat smaller than the surrounding accessory cells, and contain dark opaque material. The stomata open into the intercellular spaces of the assimilating tissue.

Under the epidermis is the assimilating tissue composed of a single layer of elongated palisade-like cells and many intercellular spaces. In some sections (fig. 8) these palisade-like cells form a continuous layer under the epidermis, but more often the layer is interrupted on the adaxial side by fibrous cells of the bundle sheath (fig. 10). Frequently the cells are filled with dark organic material. Interiorly the assimilating tissue abuts the bundle sheath.

The bundle sheath is a conspicuous feature. As compared with the entire section it is a massive tissue made up of several layers of large, thick walled cells, isodiametric in cross section and much elongated in longitudinal section. Some of the cells are filled with light or rarely dark brown material but most of them lack any contents. In some of the sections (fig. 10) the sheath is seen not only as a continuous layer about the vascular strand, but on the adaxial side it is expanded and extends to the epidermis.

The vascular bundle occupies the center of the leaf. Figure 12 represents a bundle in which the preservation is almost perfect and shows the distribution of the various elements. Toward the center are lignified cells of small caliber, the protoxylem, surrounded by metaxylem interspersed with parenchyma. By far the greater development of the metaxylem is on the adaxial side; that is, it is centripetal. Between the centrifugal metaxylem and the bundle

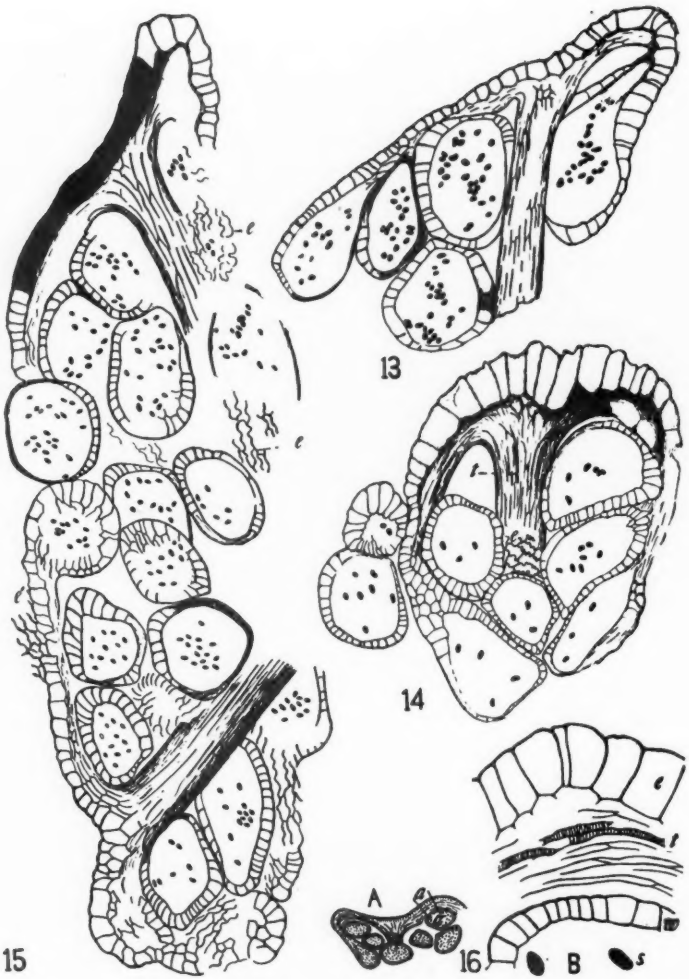
sheath are a few layers of thin walled, irregular, and somewhat crushed cells which doubtless constitute the phloem; the bundle is therefore interpreted as being collateral and not concentric, as THOMAS reported for *Calamites* leaves (3). Surrounding the conducting strand but chiefly developed laterally are thin walled parenchymatous cells.

SPORANGIA

As almost every section of the coal ball contained leaves of the type just described, so also on almost every section might be found groups of sporangia, sometimes few in a cluster and sometimes many, some apparently detached and isolated and others still in connection with the sporangiophore. The position of the sporangia with reference to the sporangiophore and the juxtaposition of two of the sporangiophores (fig. 15) strongly suggest an original aggregation of these organs into a cone. The peltate top of the sporangiophore and the mode of attachment of the sporangia are Calamitean and Equisetalean.

The sporangiophore consists of a long stalk which expands distally into a peltate top. The edge of the peltate top is curved under and divided into lobes, each lobe more or less extended and bearing on the end a single sporangium (figs. 13, 14). The number of lobes and corresponding number of sporangia attached to a single sporangiophore were not definitely determined, but following through a single cluster for little more than half its thickness eighteen sporangia were counted. Twenty-five to thirty sporangia to a sporangiophore, therefore, would be a rough but conservative estimate.

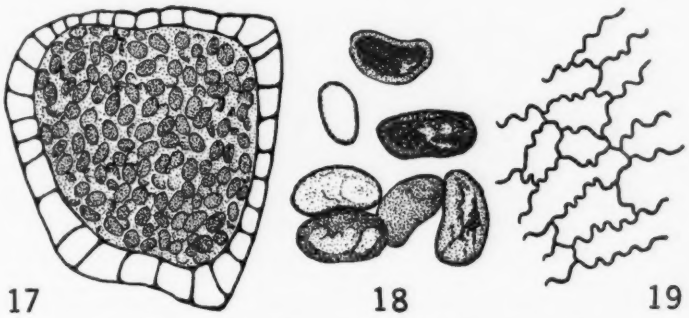
The stalk is composed of narrow, elongated, thick walled cells inclosing a central vascular strand. In section the upper epidermis of the scale presents a columnar or palisade-like layer, underneath which the cells are smaller and radially elongated; in this last tissue may be found tracheids which are directed toward the sporangia (fig. 16). The scale is thickest toward the center and gradually tapers to the edge, where it narrows to the two epidermal layers with few, perhaps not more than three or four, cells in between (fig. 14); then it is as if the two layers expand again and become the wall layers of the sporangia (figs. 13, 14, 15). Surface views of the epi-



FIGS. 13–16.—Figs. 13, 14, 15, sporangiophores and sporangia: *e'*, surface view of few of epidermal cells of peltate top of sporangiophore; *e*, surface view of epidermal or wall cells of sporangia. ×35. Fig. 16, *A:* diagram of sporangiophore and sporangia. *B:* detail from *a–a* of *A* showing: *t*, vascular elements of peltate top leading to sporangium; *e*, epidermis of peltate top; *w*, wall of sporangium; *s*, spores. ×95.

dermal cells are sometimes found (figs. 15, 19); except for their position it would be difficult to distinguish between the epidermal cells of the scale and a surface view of the cells composing the wall of the sporangium (fig. 15).

The sporangia are round to angular in section (figs. 13, 14, 15), the latter character obviously resulting from their large number and the compact arrangement. As preserved, the sporangial wall is but one cell thick with no trace of tapetal tissue. The spores are elongate-



FIGS. 17–19.—Fig. 17, section of sporangium with spores. ×94. Fig. 18, detail of spores. ×366. Fig. 19, surface view of epidermis of peltate top of sporangiophore. ×94.

elliptical, 50 μ long and about 25 μ wide. The spore coats have no characteristic markings. They are smooth save for a slight wrinkling, and are devoid of contents.

Discussion

In the stem fragment the carinal canal, centripetal protoxylem, and verticels of leaf sheaths make a combination of characters found among Paleozoic plants only in the Calamitaceae. The arrangement of the secondary wood and rays is conformable with that of *Calamites* (2). Grouping of carinal canals in pairs has been reported in the axis of the strobilus of *Palaeostachya vera*, a cone referred to *Calamites* (1); yet the particular type of grouping found in this specimen is a new feature. In addition, the relatively small size and inconspicuousness of the carinal canals, together with the massive pith consisting of thick walled, almost fibrous, cells, are features

specific to the specimen. These are of sufficient importance to warrant the establishment of a new species, for which the name *multifolia* is proposed.

Calamites multifolia sp. nov.

SPECIFIC DIAGNOSIS.—Stem hollow with fistular cavity surrounded by extensive pith of relatively thick walled cells which toward the center is arranged in wedge-shaped groups. Carinal canals between forty and fifty, small and inconspicuous, disposed in groups of four with the two lateral canals of each group on shorter radii than the ones in between.

In the kind and distribution of their fundamental tissues there is such close agreement in the leaves just described with those of the *Asterophyllites* (*Calamocladus*) type that there seems no doubt of their generic position. In the detail of structure, however, there are specific characters, consisting in the amount of development of the several tissues and in the arrangement of vascular elements, which serve to set these leaves apart into a separate species for which the name *multifolia* is again employed.

Asterophyllites (*Calamocladus*) *multifolia* sp. nov.

SPECIFIC DIAGNOSIS.—Leaves acicular, 0.8 mm. wide by 0.45 mm. thick, more than 6 mm. long. In transverse section roughly rectangular. Center traversed by a single collateral mesarch bundle surrounded by a bundle sheath of fibrous cells which on the adaxial side may extend to the epidermis. Assimilating tissue of elongated palisade-like cells and intercellular spaces. Stomata lateral and superficial.

The fragmented condition of the sporangiophores and the fact that there is only an indication of their aggregation into a strobilus make their assignment to any known fructification impossible. The peltate scale and the origin and attachment of the sporangia are definitely Equisetalean; but the large number and small size of the sporangia are at variance with any of the recorded genera of extinct forms, in which the usual number of sporangia attached to a sporangiophore is four, which extend from the peltate scale to the axis of the strobilus. With the information at hand, however, it does not

seem wise to establish a new genus for the reception of these clusters of sporangia; hence they are simply recorded as new fructification fragments possessing Calamitean characters.

Summary

1. Fragments of a stem, leaves, and sporangiophores bearing sporangia containing spores, all hitherto undescribed species and all possessing Calamitean characters, were found in coal ball 236 of the Harrisburg, Illinois, collection. The geological age of the material is the Alleghany group of the Upper Pennsylvanian.

2. In its anatomical structure the stem is conformable with the genus *Calamites*, but varies in detail from any of the described species, and so the specific name *multifolia* has been assigned to it.

3. Many sections of Calamitean leaves were found, some of them remarkably well preserved. In general they resemble leaves of the *Asterophyllites* type of the British Coal Measures, yet differ from them in specific details to such an extent that they too have been assigned to a new species, *multifolia*.

4. The sporangiophores are all detached from any vegetative structure and are separated from one another, except in one instance where two of them were found so related as to suggest an original aggregation into a strobilus. They are unique in the number (twenty-five to thirty) of sporangia they bear. The sporangia are round to angular in section, with a single layer of wall cells, and contain numerous spores. The spores are elliptical to elongate, $25 \times 50\ \mu$, with smooth spore coats and devoid of contents.

5. It is suggested that the fragments described may possibly be vegetative and reproductive portions of the same plant.

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NITROGEN NUTRITION AND NICOTINE SYNTHESIS IN TOBACCO[1]

RAY F. DAWSON

(WITH ONE FIGURE)

Introduction

The influence of nitrogen nutrition on the synthesis of nicotine constitutes an important aspect of the physiological relationships of this alkaloid in the tobacco plant. THATCHER, STREETER, and COLLISON (9), and GARNER *et al.* (7) have shown that the nicotine content of field-grown plants increases with increased application of nitrogenous fertilizers. Likewise the relation of form of nitrogen to the nicotine content and quality of tobacco leaf has been studied by BAILEY and ANDERSON (3), and GARNER *et al.* (7). BEAUMONT *et al.* (4) have pointed out the lack of uniformity in results obtained in studies of the latter type, and have presented data to show that ammonium nitrogen in water culture solutions induced a higher concentration of nicotine in the leaf tissues of Havana Seed tobacco than did nitrate nitrogen. To the extent that it seems to bear on the problem of nicotine formation in the plant, this observation invites further investigation.

In the present study, the influence of ammonium, nitrate, and urea nitrogen was determined upon the nicotine content of tobacco leaves. Most of the studies thus far reported have been conducted in the field under favorable light conditions. The present investigation was conducted under opposite conditions, in which light may have been a limiting factor through its effects upon carbohydrate synthesis; that is, the plants were grown and harvested during that portion of the year in which the sun's rays are normally reduced in intensity and altered in quality. The effects of the shortened photoperiod were avoided to some extent by the use of supplementary artificial illumination. Under such conditions, between October 7,

[1] Contribution from the Osborn Botanical Laboratory, Yale University. The writer wishes to acknowledge the helpful advice and criticism of Professor CARL G. DEUBER.

1936 and March 28, 1937, three crops of tobacco were grown in the greenhouse in sand cultures provided with continuously flowing nutrient solutions.

Investigation

METHOD OF CULTURE.—The technique of sand culture with continuously renewed nutrient solutions as developed by SHIVE and co-workers (5) for use with the tomato plant was adapted for the present study. Six to 8 weeks old seedlings of the desired variety of tobacco were transplanted to glazed stoneware crocks, each of which contained 2.2 liters of clean-washed white quartz sand of 42 per cent porosity. Each crock contained a hole in the bottom for drainage. Nutrient solution dripped from capillary tubes into small glass wells imbedded in the sand to prevent surface growth of algae. Three such capillary tubes were connected to one solution reservoir by a siphon so arranged that a constant head of water was maintained regardless of the level of the solution in the reservoir. Twice daily the drip rate was adjusted by means of screw clamps. Ordinarily the rate of flow was regulated between one and two liters per day, depending upon the size of the plants and the changes in pH of the outgoing solution.

Usually, twenty-four plants were grown under these conditions for periods ranging from 5 to 6 weeks, during which time the pH of the solutions did not vary over ± 0.2. In all, four culture solutions were employed, three of which were identical with those employed by CLARK (5) in his study of the tomato. The fourth solution was molecularly equivalent to the nitrate solution of CLARK but contained instead organic nitrogen in the form of urea. The partial volume-molecular concentrations of the various solutions are given in table 1. Iron was supplied by scattering crystals of iron potassium tartrate upon the surface of the sand at weekly intervals. Manganese, boron, and copper were added in concentrations of two parts per million, and unless otherwise stated, each solution was adjusted to a reaction of pH 6.8 with sodium hydroxide. All solutions were made up with tap water from the New Haven water supply. Analyses furnished through the courtesy of the New Haven Water Company indicated that this water contained approximately 22 parts of calcium, 13 parts of sulphate, and 1.6 parts of nitrate per million.

The entire assembly of plants and apparatus was placed upon specially constructed wooden platforms on the north bench of the greenhouse. In order to counteract the effects of occasional bright days, which sometimes caused wilting, a white cheesecloth shade was erected under the roof of the greenhouse and allowed to remain throughout the duration of the experiments. The cloth was of a thin grade with a coarse mesh, which gave uniformly diffused illumination, while, according to readings obtained with a Weston light meter, it was effective in decreasing the light intensity by nearly 35

TABLE 1

COMPOSITION OF SOLUTIONS EMPLOYED IN SAND CULTURE EXPERIMENTS

Salt	Partial volume-molecular concentration			
	Nitrate	Ammonium (concentrated)	Ammonium (dilute)	Urea
Calcium nitrate	0.0042*			
Potassium monophosphate	0.0021	0.0021	0.0021	0.0021
Magnesium sulphate	0.0042	0.0042	0.0042	0.0042
Ammonium sulphate		0.0042	0.0021	
Calcium chloride		0.0042	0.0042	0.0042
Urea				0.0042

* The final partial molecular concentration of the nitrate solutions was later discovered to be less than this value, since the calculations had been made on the basis of the anhydrous salt rather than $Ca(NO_3)_2 \cdot H_2O$.

per cent on a cloudy day. Additional light was provided every evening for about 4 hours by a series of 150 watt Mazda lamps suspended 3 feet from the growing tips of the plants. These lamps were controlled by an automatic time switch. The temperature of the house was maintained as nearly as possible at 25° C., and a relatively high humidity was maintained by frequent watering down of the concrete floor.

SAMPLING, ANALYSIS, AND EXPRESSION OF DATA.—After harvesting, the midribs were removed from the leaf blades. The latter were then placed in a hot air oven for 2 hours at 80° C. All analytical data were expressed upon this dry material as a basis.

Analyses for nicotine were made according to the silicotungstic

acid precipitation method after distillation from alkaline solution with steam (2).

In the present study the data were expressed as percentage of dry weight and as absolute amount per plant. Emphasis was given the percentage method, however, in view of the fact that a knowledge of the quality of tissue formed under the influence of varying types of nitrogen nutrition represented essentially the fundamental objective in this investigation.

Influence of ammonium nutrition upon nicotine formation in leaves of maturing Turkish tobacco plants

A comparison of the effects of ammonium, urea, and nitrate nitrogen upon nicotine formation in maturing Turkish tobacco plants constituted the first phase of this study. Two concentrations of ammonium nitrogen were employed. One was molecularly equivalent to the nitrate and urea solutions; the second was equivalent to one-half the molecular concentration of these solutions, and was included to approximate a more ideal nutrient medium under the existing conditions of restricted carbohydrate synthesis than would theoretically be afforded by the more concentrated solution of ammonium salts. A brief characterization of the experimental procedure and results follows.

On November 14, approximately 5 weeks after initiation of the cultures, four groups of eight plants each were harvested and prepared for analysis. At that time, those plants supplied with nitrate (hereafter referred to for convenience as nitrate plants) were considerably taller than the plants of the remaining groups, and their leaves were a lighter green color. Likewise the dimensions and the dry weights of the nitrate plants greatly exceeded the corresponding values for the plants of all other groups.

No significant difference was observed, either in development or in dry weight, between the plants cultured in the two ammonium series (hereafter referred to as dilute ammonium plants and concentrated ammonium plants respectively). Compared with the nitrate plants, however, both groups of ammonium plants were less

well developed and were lower in dry weight. The concentrated ammonium plants possessed characteristically rugose, glossy, dark green leaves. The plants grown with urea nitrogen (designated hereafter as urea plants) were the smallest in all dimensions and the lowest in dry weight. The leaf color contrasted sharply with that of the ammonium plants in being a light yellow green. Data for this experiment are given in table 2.

The relative quantities of nicotine per unit of leaf tissue were identical in the nitrate and concentrated ammonium plants and in

TABLE 2

EFFECTS OF FORM OF NITROGEN ON NICOTINE CONTENT OF TURKISH TOBACCO LEAVES

FORM OF NITROGEN	DRY WEIGHT OF LEAVES PER PLANT (GM.)	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT (MG.)
Nitrate	5.83	1.26	73
Ammonium (concentrated)	3.35	1.28	43
Ammonium (dilute)	3.33	1.05	35
Urea	2.23	1.04	23

the dilute ammonium plants and urea plants respectively. The value for the first two was approximately 20 per cent above that for the latter two groups.

A number of apparently significant observations arise from the data just outlined. In the first place, the proportion of nicotine per unit of dry leaf tissue was the same for both the nitrate and the concentrated ammonium plants. Such a result was not in accord with the findings of BEAUMONT *et al.* (4), who reported that ammonium nitrogen nutrition definitely increased nicotine content per unit of mass. In the present case, nicotine concentration in the leaves seemed to be correlated with quantity rather than with quality of nitrogen supplied to the roots.

In the second place, the nicotine content of the leaves of the dilute ammonium plants was 15 per cent below that of the leaves of the concentrated ammonium plants, although their respective dry weights were practically identical. The only apparent conclusion

seems to be that the dilute ammonium solution represented a concentration of nitrogen which quantitatively limited nicotine formation.

On the other hand, leaves of the urea plants, although attaining only about two-thirds the dry weight of the dilute ammonium plants, yet possessed the same percentage content of nicotine. It was evident throughout the course of the experiment that urea was inferior to either ammonium or nitrate as a nitrogen source. This result is in contrast to the reported findings of others (1, 4) in which urea employed in water cultures and in field experiments gave results intermediate between ammonium and nitrate, or even superior to both, as regards plant growth.

Influence of ammonium nutrition upon nicotine synthesis in leaves of immature Havana Seed tobacco plants

A second phase of the study was concerned with the effects of nitrate and ammonium nutrition upon the nicotine content of immature tobacco plants of the Havana Seed variety under conditions unfavorable to the elaboration of carbohydrates. This experiment was begun on November 19, 1937, at which time the plants were about 10 cm. in height and bore from three to four leaves each. The methods employed were exactly the same as for the previous experiment, except that the nitrate solutions were left at their own pH of 6.4. The ammonium solution used was the concentrated solution of the previous experiment. The plants were placed on the north bench in the greenhouse where they were exposed to reduced light and temperatures. As a result, growth was very poor, and at the end of 23 days the plants had put forth only three or four new leaves each. The plants were harvested and dried at this point.

In contrast to the previous experiment, a sizable increase in the percentage of nicotine was found in the ammonium leaf tissues. Furthermore, the absolute nicotine content of the ammonium leaf tissues showed the same quantitative increase when based on the number of grams per plant. The nitrate plants developed the typical light green leaf color already mentioned in connection with the previous experiment, while the ammonium plants possessed glossy, dark

green, rugose leaves with a much greater water content. Table 3 gives the dry weight and nicotine values for this experiment.

The increased percentage of nicotine in the ammonium plants is perhaps not out of harmony with the results of other studies, since it has been rather conclusively shown that nicotine synthesis is influenced to some extent by the level of nitrogen nutrition (**7**), also that ammonium nitrogen is assimilated by plants in the earlier stages of development much more readily than nitrate nitrogen (**8**). If there is any relationship between these observations and the results obtained in the present study it probably lies in the early stimulation of the nicotine synthetic process, due to an increased assimilation of nitrogen in the ammonium plants rather than to a sustained higher rate of nicotine formation from the beginning of the experiment. In any case, the main conclusion seems to be that in the early stages of growth, even under conditions essentially unfavorable for carbohydrate synthesis, the formation of nicotine proceeds at a more rapid rate in tobacco leaves under the influence of ammonium nitrogen than under the influence of nitrate nitrogen.

TABLE 3

EFFECTS OF AMMONIUM AND NITRATE NITROGEN ON NICOTINE CONTENT OF HAVANA SEED TOBACCO LEAVES

FORM OF NITROGEN	DRY WEIGHT OF LEAVES PER PLANT (GM.)	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT (MG.)
Nitrate	0.69	0.92	6
Ammonium	0.74	1.33	10

INFLUENCE OF AMMONIUM AND NITRATE NUTRITION AND SOIL CULTURE UPON NICOTINE FORMATION IN CONNECTICUT BROADLEAF TOBACCO DURING THE PERIOD OF MOST ACTIVE GROWTH

The difference in results obtained between the last two experiments suggested the desirability of still further investigation designed to follow the changes in nicotine content throughout the period of most active growth of the plant under conditions of ammonium and of nitrate nutrition and of limited light. It was hoped

to learn whether or not there is an initial rapid synthesis of nicotine in ammonium culture followed by a gradual decline in rate of formation during the later stages of growth. It was further considered desirable to include as controls a similar set of plants grown in soil in order that the differences between the nitrate and ammonium cultures might be more fully evaluated. The plants were placed under experimentation when 70 days old and were harvested in four crops, beginning at the eighty-eighth and extending to the one-hundred-twelfth days. The last sample was collected on March 28.

TABLE 4

EFFECTS OF AMMONIUM, NITRATE, AND SOIL CULTURE UPON NICOTINE CONTENT OF CONNECTICUT BROADLEAF NO. 38 TOBACCO LEAVES

Days from seed	Dry weight of leaves per plant (gm.)			Nicotine percentage			Total nicotine per plant (mg.)		
	NO_3	NH_4	Soil	NO_3	NH_4	Soil	NO_3	NH_4	Soil
88	1.3	0.9	1.4	1.05	1.39	2.03	14	13	28
99	3.4	3.3	3.1	1.04	0.98	1.47	35	32	46
108	7.6	5.4	7.9	0.90	0.90	1.28	68	49	101
112	11.5	7.0	9.4	0.88	0.89	1.50	101	62	141

Twenty-four plants of the Connecticut Broadleaf no. 38 variety were employed, twelve each in nitrate and ammonium culture. The conditions of lighting, the composition and rate of flow of the nutrient solutions, and the temperature and humidity of the greenhouse were as nearly as possible adjusted to duplicate the conditions employed in the first of these experiments. At the time of transplanting the seedlings to sand, a number of uniform individuals were also transferred to 8 inch pots of soil, where they were cultured as described earlier in this paper. The sand employed was not the white quartz sand previously used but was a yellow sea sand obtained from Cow Bay, New York, which had an average porosity equal to that of the white quartz sand, but which had in addition a much wider range of particle size. Measurement of both sands with standard mesh sieves indicated a very uniform porosity of 42 per cent for the white sand and an average porosity of 42 per cent for the yellow

sand, with a range for the latter between 40 and 44 per cent. This sand was thoroughly washed with running water until free from fine debris before using.

Growth in the ammonium series followed a very regular course, in contrast with the more normal sigmoid type of growth curve of the soil and nitrate plants. It is likely that the final flattening off of the curve (fig. 1) for the soil plants was due to root binding within

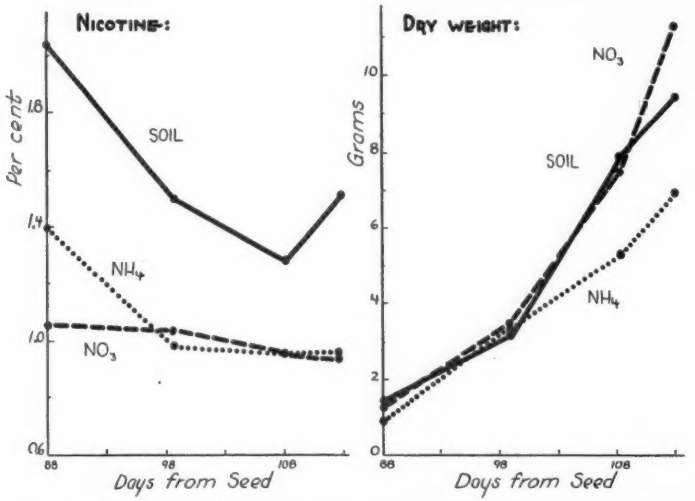

FIG. 1.—Effects of culture in sand with ammonium and nitrate nitrogen and of soil culture upon dry weight and nicotine content of Connecticut Broadleaf tobacco leaves.

the clay pots, since growth of both soil and nitrate plants had been very similar up to that point.

The nicotine content of the leaves expressed as percentage of dry weight presented some interesting variations (fig. 1). In the first place, the observations of earlier experiments seem to have been completely upheld in regard both to the initial rise in nicotine content under the influence of ammonium nitrogen and to the final equivalence of the nicotine values for both ammonium and nitrate plants. The data bring out further details of these relationships, however, which have not been made evident in the preceding experi-

ments. In both cases those plants in the sand cultures possessed a higher percentage of nicotine in their leaf tissues at the time of first sampling than they did at later samplings, and the decrease in percentage values was fairly uniform from the first down to the last sample. Furthermore, inspection of each corresponding pair of decreasing values reveals that the relative nicotine contents of ammonium and nitrate leaves were very nearly the same in each sample after the first. Nicotine in the leaves of the soil plants had attained a concentration considerably in excess of that in the leaves of the ammonium and nitrate plants at the time of first sampling. Subsequently the nicotine content of the soil plants decreased irregularly until, at the time of last sampling, it had reached a value nearly 1.7 times that of the final values for the ammonium and nitrate plants. In the absence of further data, it may be suggested that the increased nicotine content of the leaves of the plants in soil was associated with a hydration factor (**6**).

Summary

1. The influence of ammonium, nitrate, and urea nitrogen upon nicotine synthesis in tobacco leaves has been investigated under conditions unfavorable for optimum carbohydrate formation.

2. Ammonium nitrogen compared with nitrate seemed definitely to have increased the relative nicotine content of tobacco leaves in the earlier stages of growth. This influence was soon lost, under the conditions of the experiments, and the ammonium-cultured plants, although lower in dry weight and showing definite indications of ammonium toxicity, subsequently possessed nicotine concentrations indistinguishable from those attained by the nitrate-cultured plants of the same age.

3. No essential difference was observed between the effects of "concentrated" and "dilute" ammonium nitrogen upon plant growth and dry weight. However, the nicotine content responded to the increased nitrogen supply of the concentrated ammonium solutions by assuming somewhat higher proportions in the leaves.

4. Growth of plants supplied only with urea nitrogen was inferior to the growth of plants cultured in ammonium solutions, either at concentrations of ammonium equivalent to or half that of the urea.

The influence of urea on nicotine percentage was equivalent to the influence of one-half the same molecular concentration of ammonium salts.

5. Solution cultures, regardless of the form of nitrogen supplied, induced a lower concentration of nicotine in the leaves than did soil cultures even though the dry weights of the leaves were the same. Such an action may have been associated either with differences in the moisture content and aeration of the culture medium or with qualitative or quantitative differences in the salt composition of the culture solutions.

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HISTOLOGICAL RESPONSES OF CABBAGE PLANTS GROWN AT DIFFERENT LEVELS OF NITROGEN NUTRITION TO INDOLE(3)ACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 495

ETHEL GOLDBERG

(WITH TEN FIGURES)

Introduction

Considerable histological work has recently been done on the responses of various plants to applications of growth promoting substances. FISCHNICH (3) worked with *Coleus;* KRAUS, BROWN, and HAMNER (9), HAMNER and KRAUS (7), and LINK, WILCOX, and LINK (11) with bean; BORTHWICK, HAMNER, and PARKER (2) with tomato; HARRISON (8) with *Iresine;* and HAMNER (6) with *Mirabilis.* All reported great stimulation of various tissues, resulting in the production of callus and root primordia. GREENLEAF (5) noted the production of shoots from the cut surface of *Nicotiana* treated with indole(3)acetic acid, but reported no histological work. BEAL (1) made a detailed study of bud development from leaf axils in *Lilium harrisii* induced by applications of indole(3)acetic acid to the cut surface of the decapitated stem.

Cabbage was found to be very responsive to indole(3)acetic acid, and to produce shoots from the decapitated surface of a large proportion of treated seedling stems (4). It was also noted that the reaction of the plants, at least in rate, to applications of this substance was largely dependent upon the nutritive state of the plant. These observations were made the basis of the anatomical study reported here.

Material and methods

About 2000 young cabbage plants of the Wisconsin All Seasons variety were used throughout the experiments. All were grown under ordinary greenhouse conditions of light and humidity. Some were grown in soil, while others were grown in sand in the Chicago

soil-temperature nutrient tanks, which maintain the root temperature at 20° C. with a variation of only 1° in either direction. There was an average diurnal range in greenhouse temperature of 13° C. (24° to 37° C.). The plants in sand were watered each day with either Shive's R_2S_5 nutrient solution, or the same solution in which the calcium nitrate had been replaced by calcium chloride.

A 3 per cent mixture of indole(3)acetic acid (Merck) in lanolin was used for all applications. Material for histological work was collected at intervals of 6 hours for the first 72 hours; every 12 hours for the next 144 hours; and every 24 hours thereafter until 504 hours had elapsed. It was fixed in Navashin's solution and imbedded by the butyl alcohol-paraffin method. Sections were cut at 12 μ and stained with triple stain.

Experiments and results

Seedlings grown in soil

Decapitated first internodes.—Seedlings 20 days old grown in ordinary greenhouse soil were cut squarely across near the top of the first internode. The cut surface was then smeared with the indole(3)acetic acid mixture. Controls were smeared either with lanolin or were left with no treatment other than decapitation.

At the time of treatment the stems had just begun to undergo secondary thickening (fig. 2*A*). A well defined epidermis and cortex have developed, the outermost cells of which contain abundant chloroplasts. The endodermis can be distinguished from other cortical cells, although with difficulty, by the denser cytoplasm and somewhat more prominent nuclei. Sieve tube and companion cells of the primary phloem are mature, and one or two rows of pericyclic cells still showing protoplasmic content cap each phloem group. The cambium is just beginning its activity, with not more than two rows of as yet undifferentiated cells on either side. Prominent xylem rays are apparent in some of the larger bundles. The pith is large in extent, with comparatively narrow medullary rays passing between the bundles.

Within 18 hours after treatment, cell enlargement in the pith is apparent. By the end of 24 hours, the cortical cells also show an

enlargement. After 54 hours the size of these cells has almost doubled, and epidermal cells have also enlarged to keep pace. This is the maximum enlargement of individual cells of the pith; occasionally some cortical cells in later stages may almost triple their size.

By the end of 30 hours an increase in the activity of the cambium and a delay in maturation of its derivatives are apparent. The endodermis is much more easily identified, owing to the increased density of the cytoplasm and prominence of its nuclei. Many of the phloem parenchyma cells enlarge, and these, together with primary xylem parenchyma, rays, and a few pith cells adjacent to the protoxylem points, show dense cytoplasm and very prominent nuclei. An increased density of protoplasm is also apparent in some of the pericyclic fibers, but it is doubtful whether they play any part in further activity within the stem.

First divisions in the endodermis appear in 42 hours. These may be in any plane, and within 48 hours some cells have undergone division into three, while others are multinucleate. The outermost cells of the medullary and bundle rays divide rapidly and their derivatives increase in size until they push aside the inactive pericyclic cells. There is some division in the primary phloem and the cortex.

Within 60 hours the sites for development of later root primordia have been determined owing to the extremely rapid division of the ray cells and the somewhat less rapid, but nevertheless marked, division of the phloem parenchyma. Certain of the endodermal cells have divided into as many as four (fig. 2*B*), which form a cap over the dividing ray cells. Pericyclic cells are dispersed and in some cases the phloem cells thus come to lie next to the endodermis. The primary phloem cells become crushed.

At the end of 144 hours the histogens of the root primordia have become organized, mainly from ray tissue flanked by phloem derivatives. A cap of endodermis 3–6 layers of cells wide is pushed outward by the growth of the root, which soon pushes through the cortex and epidermis (fig. 3*A*).

Numerous xylem elements mature, some of which differentiate back to abut against the secondary xylem cells of the stem. The xylem parenchyma proliferates to the extent that the original pro-

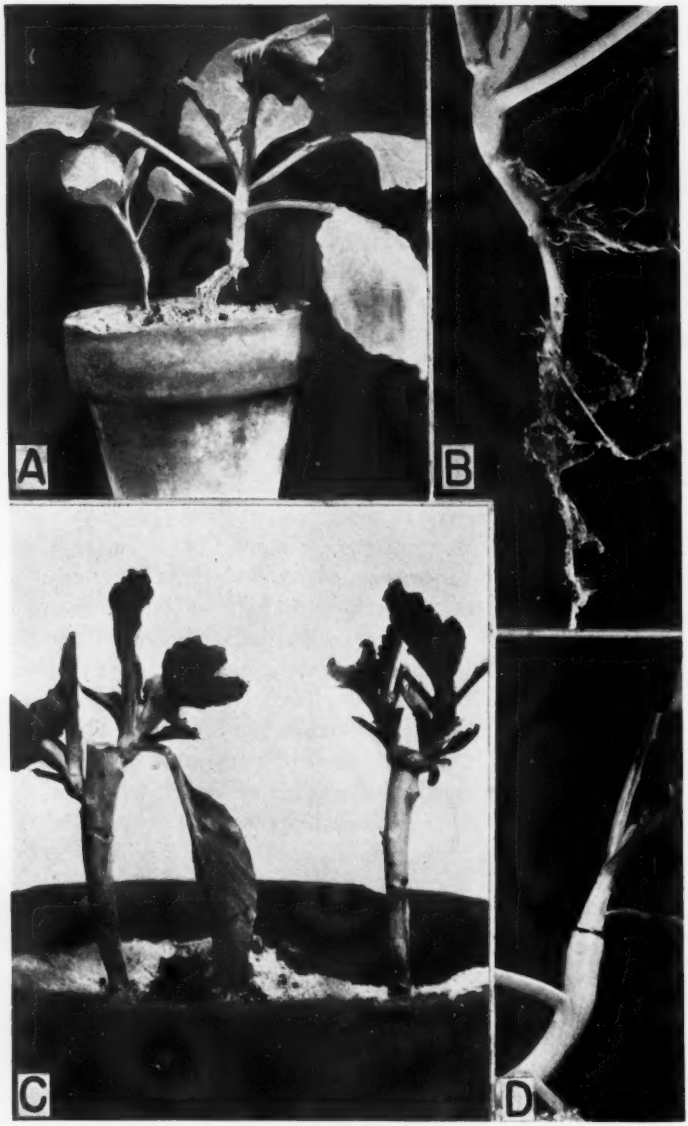

FIG. 1.—*A:* +N and −N control plants showing gross differences 1 week after treatment of other plants of series. *B:* +N plant 3 weeks after placing small daub of lanolin mixture on unwounded internode and covering with sand. *C:* decapitated plants 3 weeks after wounding; plant on left untreated; one on right smeared with lanolin mixture. *D:* unwounded internode 4 days after ringing with fine thread of lanolin mixture. Ink circle drawn on site of application just before photographing.

toxylem points are dispersed. The parenchyma of the proximal parts of the rays, together with the adjacent pith and the pith adjoining the protoxylem points, proliferate greatly, producing a confused array of vascular strands, disorganized tracheids, and highly meristematic areas which may be circular, semicircular, or straight, and oriented in any direction. The vascular strands produce mainly xylem elements with a few irregularly placed phloem cells. Large mounds of callus are usually produced, mainly by the phloem. Root primordia in large numbers are produced only in the region of the original stem, below the callus. They are usually fasciated.

The vascular tissue maturing in the callus at first differentiates downward and connects with the vascular tissue of the root primordia. Usually only disjointed groups of vascular tissue are apparent later. These may be large, or so small as to consist of only one tracheid. The large groups may have very well developed cambiums which run in any direction and then abruptly end. The whole presents a picture of extreme confusion.

About one-third of the plants investigated produced shoots as well as roots, although in such cases the number of roots was greatly reduced (figs. 3*B*, 4, 5, 6). Shoots arise either laterally from tissues outside the vascular bundles which have been stimulated by the indoleacetic acid or from the callus over the surface of the cut end. No plants were noted which produced both lateral and apical shoots. Several shoots of either kind were produced by one plant, as many as ten having been counted on an individual stem. There appears to be no functional difference between shoots produced in either position, the only difference being in point of origin. The lateral shoots orient themselves in an upright position; and since they are located so close to the apical end, after some little growth they cannot be told apart from the others except with great difficulty.

Shoots arising laterally (fig. 4) are initiated by rapid cell divisions, either in the central or innermost cell layers of the cortex or in the rays. Since they originate in the region of the stem which produces adventitious roots, in the earliest stages they cannot be distinguished from young roots except that they do not have a cap of endodermis. In their outward growth, tissues in their path are crushed, and when about to emerge through the epidermis a terminal meristem be-

comes organized. Vascular tissue differentiates back until a connection is made with that of the main stem. Soon a smooth juncture of cambiums of new and old stems comes about and the shoot continues its growth as would the original stem tip.

Shoots arising terminally involve a greater number of tissues. Most of them were found to arise from external callus produced by either the pith or the phloem (figs. 3*B*, 5, 6), and never a combination of the two. Cortex, xylem, cambium, and endodermis may also be involved with either pith or phloem to a small extent. Here, as in the lateral shoots, a complete tieup of new and old vascular tissues is effected, either directly as in figure 6, or indirectly as in figure 7. The meristematic areas seen clearly in the pith of the latter figures are connected directly with a large shoot above that level, and indirectly by means of vascular strands to the organized vascular system of the old stem below that level.

Control plants produce no mounds of callus tissue at the cut surface. On the contrary, more of the layers of pith cells beneath the cut die than do those over the other tissues, thus causing a sunken appearance in the center. Cell divisions in a horizontal plane occur, so that a weak phellogen is produced across the cut surface, which remains about as flat as at the time of decapitation.

UNWOUNDED AND WOUNDED FIRST INTERNODES.—A small daub of the 3 per cent mixture was placed on the first internodes just below the petiole of the first leaf of 28-day-old seedlings grown in soil. Care was taken not to injure the stem. Gross reactions of unwounded plants were somewhat faster than those of decapitated ones. Reaction occurred almost entirely below the point of application, very slightly above, and was not apparent laterally to any great extent. Under the application of the paste an elongated, narrow lesion appeared, which became sunken and brown, extending downward through the entire internode. Through this the more or less longitudinally regular rows of adventitious root primordia protruded by the end of 9 days. These did not elongate further under the conditions in which they were grown.

The fourth internodes of 45-day-old seedlings were encircled with an extremely fine band of the lanolin mixture. Here again the reac-

tion was limited almost completely to those portions of the stem below the site of application (fig. 1*D*). A definite swelling of the entire internode, which was first apparent within 24 hours, was soon extended downward through several internodes. Within 48 hours after treatment a pronounced whitening of the stem throughout the swollen portion occurred, followed within about a week by the production of root primordia.

Except for two features, the early stages of histological reactions were similar to those of decapitated plants. The activation of all tissues except the pith and the centripetal ends of the rays was the same as in that of decapitated plants. These two tissues, however, showed no response to the application (fig. 8*B*), and could not be distinguished from those of untreated plants. The second feature was the lack of protruding callus. If the treated part of the stem was not placed in a moist chamber, the rows of root primordia eventually became buried by a smooth mound of callus, and the lesion apparently healed over.

Seedlings grown in sand: Responses in relation to nitrogen deficiency

Seedlings 40 days old which had been grown in sand and supplied with complete nutrient solution were separated into two groups, one of which was continued on the complete nutrient while the other was given the nutrient solution which lacked nitrates. Ten days later, which was at the time of treatment, the −N plants tested negative to diphenylamine. They were lighter green in color, somewhat smaller in size, and a little woodier than the +N ones. These differences became much more pronounced with the progress of the experiment, as can be seen by figure 1*A*, which shows a control plant of each group.

Decapitated plants.—The +N plants were decapitated between the seventh and eighth nodes, while the −N ones, owing to their shorter height, were decapitated between the sixth and seventh nodes. This was as close to the top of the plant as it was possible to cut and get a smooth internode.

The general histological response pattern under both conditions of nutrition was the same, although differences were apparent in the growth rate and somewhat in the type of reaction of the different tissues involved.

At the time of treatment the average stem diameter of the −N plants was about 15 per cent smaller than that of the +N plants. The cortex of the latter was relatively wider and its pith was relatively very much larger than that of the former, the differences being ascribable to number of cells and not to cell size. Cell walls of the −N plants, especially those of the pericyclic fibers, were heavier, the relative amount of vascular tissue was much greater, and the cambial activity was less than that of the +N ones (fig. 8*C*, *D*).

By the end of 48 hours (fig. 9*A*) considerable cell divisions in the rays, primary phloem parenchyma, and endodermis, and some in the pith have occurred in the +N plants, while in the others (fig. 9*B*) the only apparent response is a prominence of nuclei in the endodermis and rays.

Within 90 hours the root primordia of the +N plants (fig. 9*C*) have been almost completely organized, while the cortex, endodermis, phloem, rays, and pith cells are all actively dividing. Mounds of callus tissue are appearing over the top of the cut surface. The tissues are about equally activated along all radii. The −N plants of this age (fig. 9*D*) are not quite so far along in development as were the +N plants in 48 hours, and their vascular cylinders are very unequally activated, large portions remaining unresponsive.

By 132 hours the ring of root primordia of the +N plants (fig. 10*B*) has pushed out so far as completely to disrupt the cortex and epidermis. An inner ring of root primordia, derived from pith cells especially opposite the pith rays, are growing inward. This is not apparent in all the plants studied, however. Many circular vascular strands are forming in the rays between the protoxylem points (fig. 8*A*). On the other hand, root primordia of the −N plants are not yet fully organized. Activity in the pith and cortex is much less pronounced, and divisions are confined mostly to rays, phloem, and endodermis (fig. 10*A*).

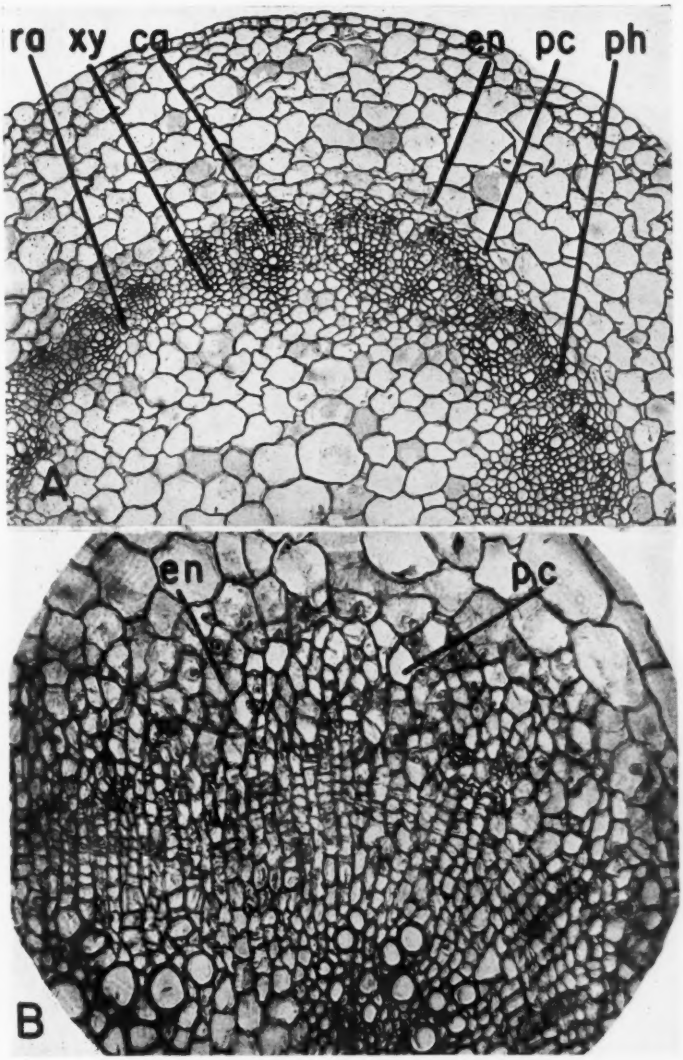

FIG. 2.—Decapitated first internodes of young plants in soil smeared with lanolin mixture. *A:* at time of treatment (*ra*, ray; *xy*, xylem; *ca*, cambium; *en*, endodermis; *pc*, pericycle; *ph*, phloem). *B:* 60 hours after treatment. Note activity in cambium, rays, endodermis, and phloem; and inactivity in pericycle.

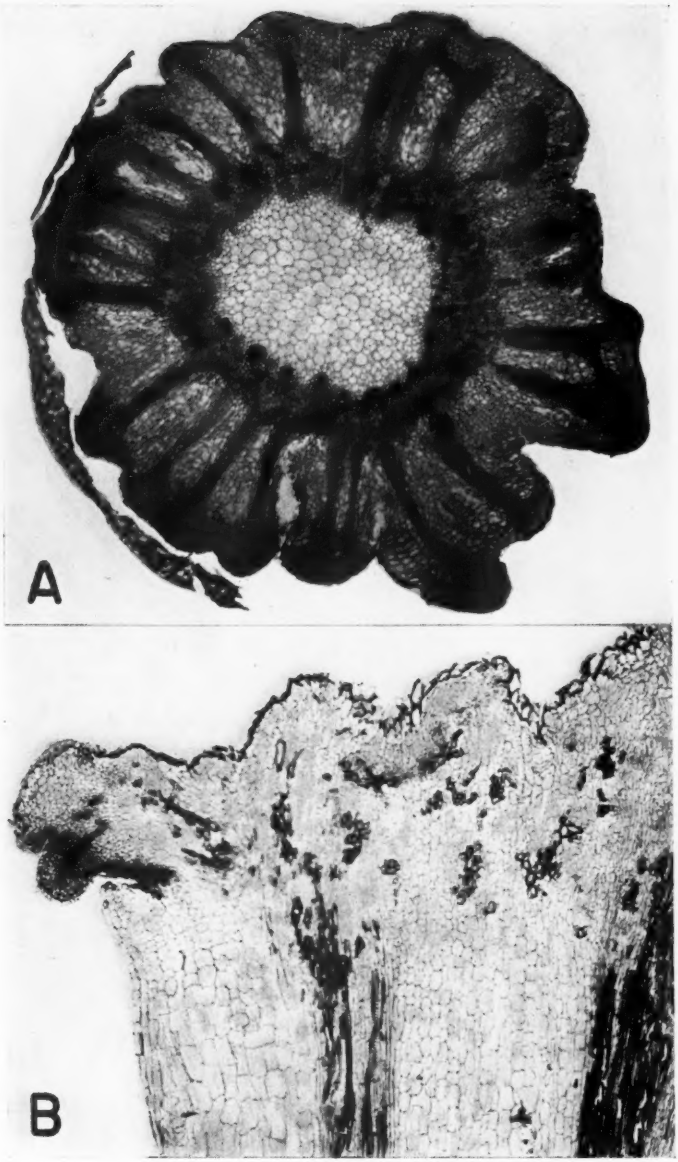

FIG. 3.—*A:* 336 hours after treatment. Root primordia pushed out through epidermis and cortex. Note activity in periphery of pith. *B:* decapitated and treated first internode of plants grown in soil, 408 hours after treatment. Terminally produced shoot involving mostly cortex, also endodermis and phloem to some extent.

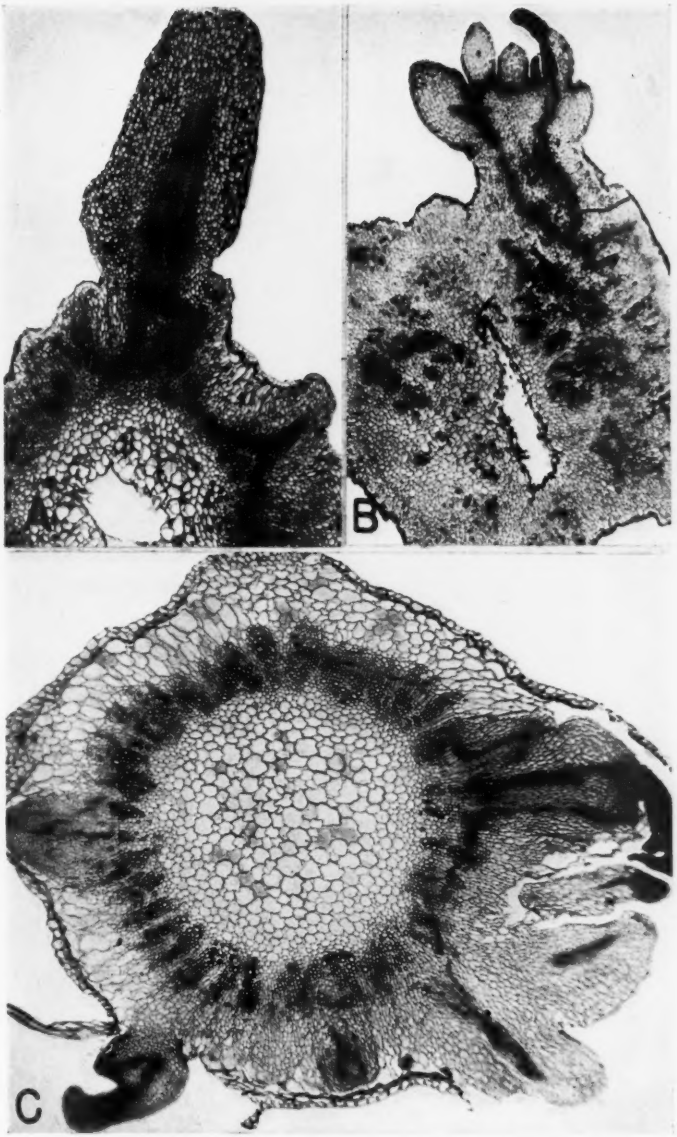

FIG. 4.—Decapitated and treated first internodes of plants grown in soil. *A:* 396 hours after treatment. Shoot produced laterally from cortex has established complete vascular connections with original stem. *B:* 408 hours after treatment. Shoot arising from callus developed over cortex has established vascular connections with vascular strands which in turn connect with vascular system of main stem. Note lack of callus developed over central part of pith and irregularity of maturation of vascular elements within callus. *C:* 396 hours after treatment. Two older shoots developed from cortex; two younger ones arising in same tissue.

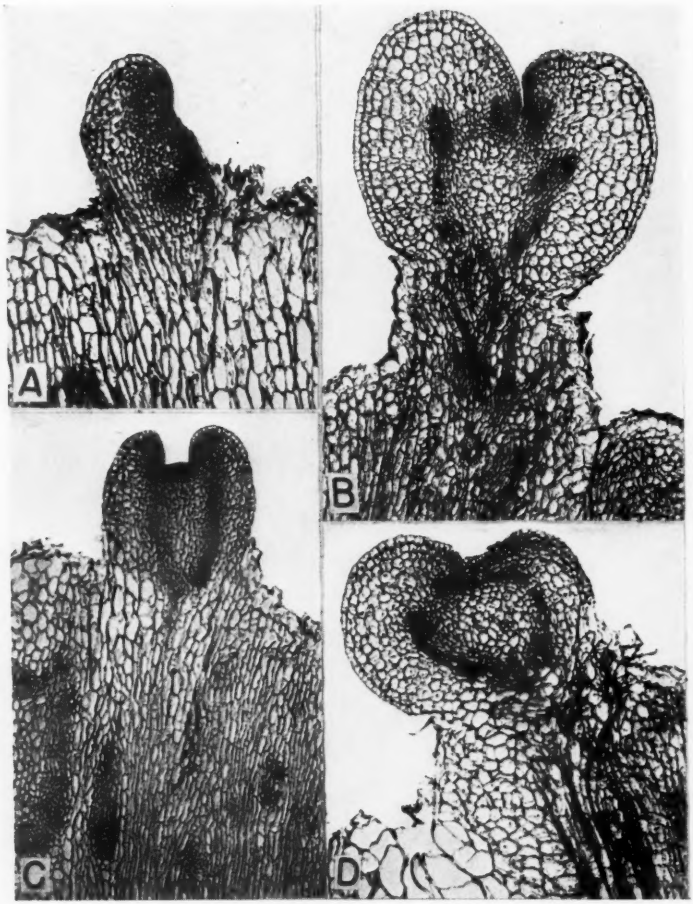

FIG. 5.—*A*, *B*, *C*, *D*: decapitated and treated first internodes of plants grown in soil. Shoots originating from callus produced over phloem.

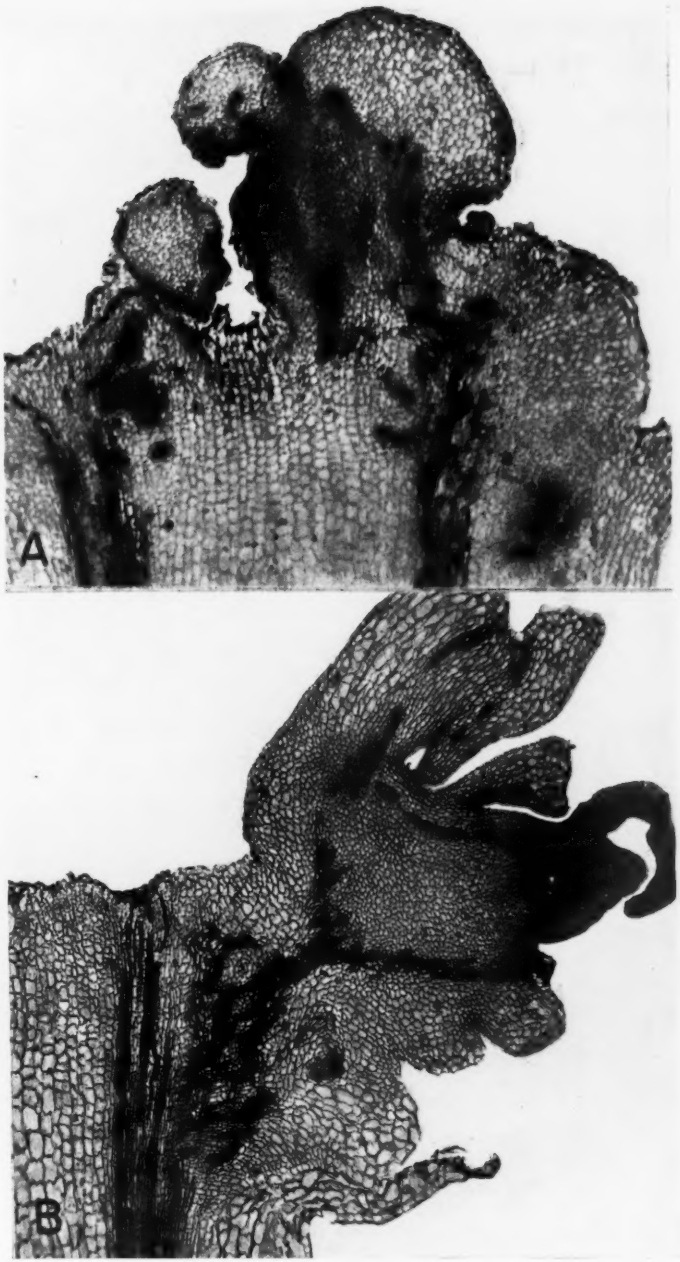

FIG. 6.—Decapitated first internodes of plants grown in soil and smeared with 3 per cent lanolin mixture. *A:* 288 hours after treatment; shoot produced from callus mostly over pith, with some involvement of xylem. *B:* 396 hours after treatment; shoot arising from phloem.

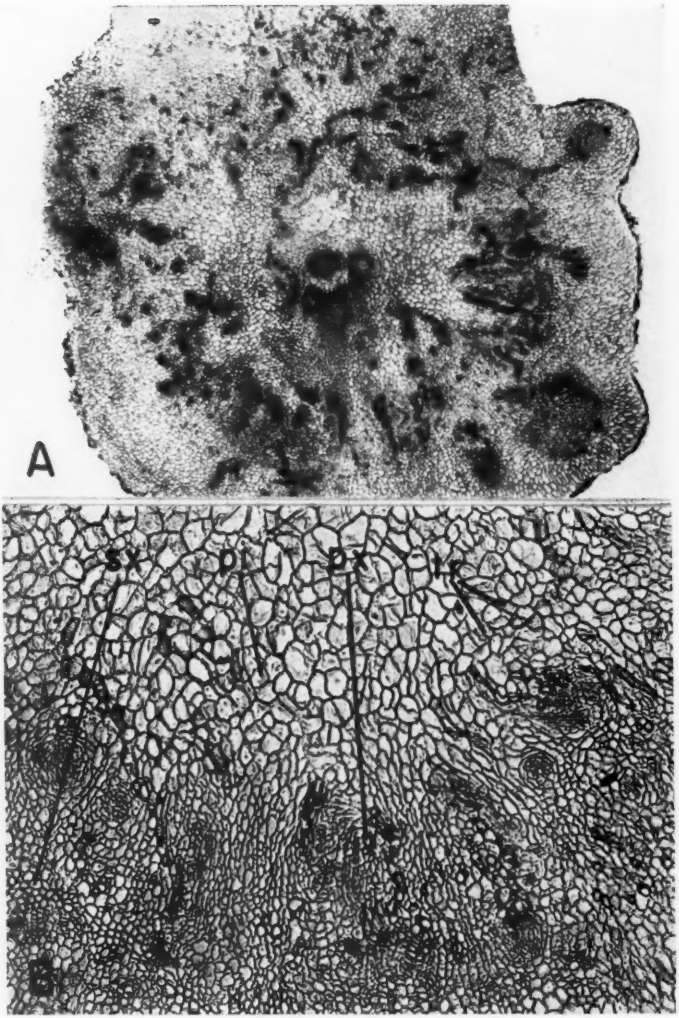

FIG. 7.—*A:* similar stem, 372 hours after treatment. Note great activity in pith, connecting above with a shoot, below with main stem. *B:* stem similar to that of *A*, one portion enlarged. Note development of vascular strands, maturation of irregularly oriented tracheids, and great numbers of cell divisions in pith.

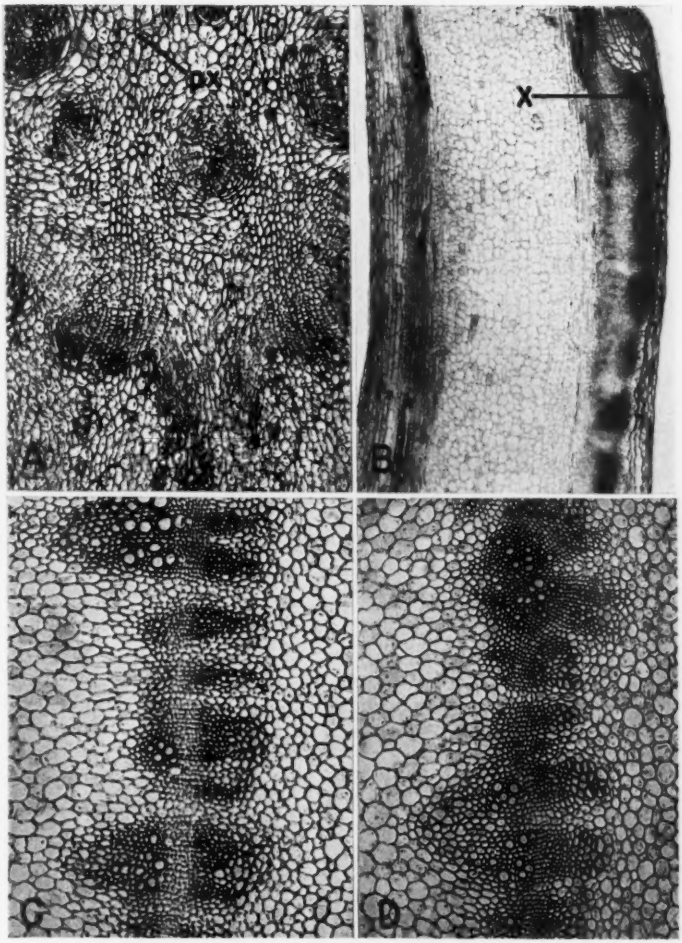

FIG. 8.—*A:* stem similar to that of fig. 7*A* and *B*, but at lower level and still further enlarged. *B:* unwounded first internode, treated by placing small daub of 3 per cent paste at *X*. Note most of activity below point of application and none on opposite side. *C:* +N plant, grown in sand, at time of treatment. *D:* −N plant, other conditions same as *C*. Diameter of entire stem of *C* actually more than twice that of *D;* therefore note lesser relative amount of vascular tissue in *C*, also its more active cambium, wider rays, and thinner walled pericyclic fibers than *D*.

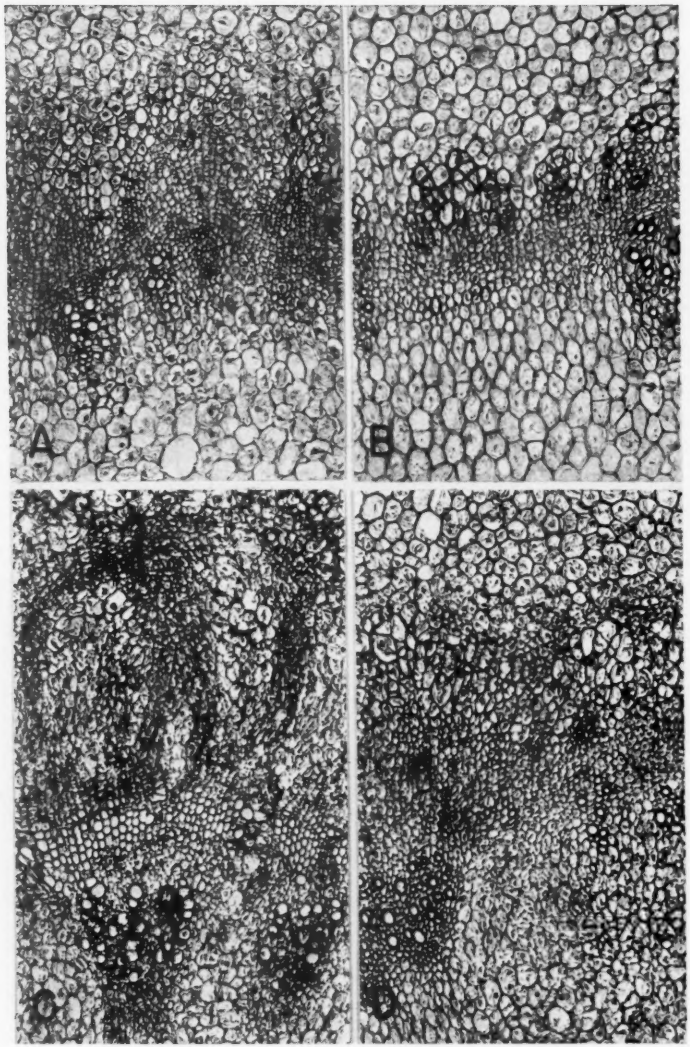

FIG. 9.—Plus N or −N plants decapitated near top of stem and smeared with 3 per cent lanolin mixture. *A:* +N, 48 hours after treatment. Great activity in rays, cambium, and phloem, some activity in endodermis and periphery of pith. *B:* −N, 48 hours after treatment. No change from time of treatment except prominence of nuclei in endodermis and rays. *C:* +N, 90 hours after treatment. Location of root primordia determined. Reactive tissues shown in *A* plus the cortex have proliferated extensively. *D:* −N, 90 hours after treatment. Large numbers of cell divisions in endodermis, rays, and cambium, some divisions in cortex and pith.

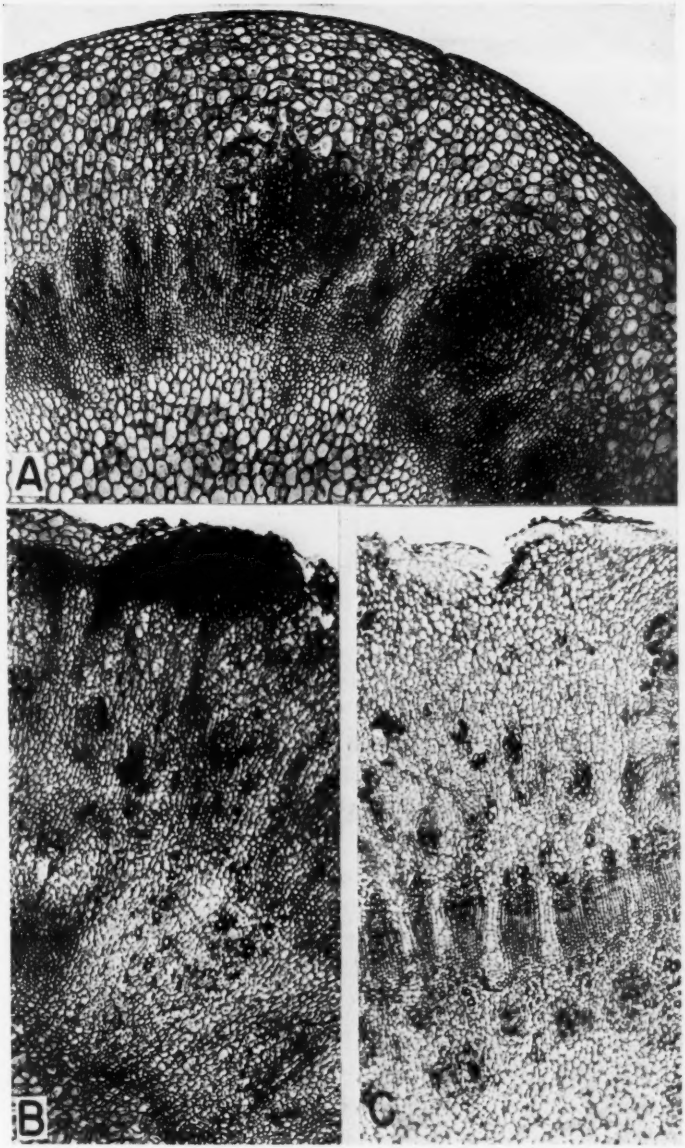

FIG. 10.—Plants similarly treated to those of fig. 9. *A:* −N, 132 hours after treatment. Root primordia just being organized. Note unequal activation of vascular cylinder and comparative inactivity of all tissues especially pith and cortex. *B:* +N, 132 hours after treatment. Note well developed root primordia, vascular strands, root primordia in pith, proliferation of xylem parenchyma which has pushed apart primary xylem elements. Epidermis and most of cortex gone. *C:* −N, 240 hours after treatment. Below level shown in *A*. Note scattered groups of fibers in cortex.

In final development (fig. 10*C*), the −N plants produce very few, irregularly placed root primordia which rarely penetrate beyond the confines of the outer cortex and epidermis. Very little callus tissue is produced and this is less disorganized than the calluses of the +N plants. The fewer meristematic patches are smaller in extent, tend to be tangential or radial rather than circular, are largely confined to the cortex and a short lateral extent in the pith; they are more regularly vascularized, producing complete vascular strands with phloem fibers, cambium, and xylem in the same relative relationship as in the original vascular cylinders.

Many patches of fibers are scattered throughout the cortex, where cortical cells have divided and their daughter cells have matured shortly thereafter. There is somewhat less tissue breakdown than is apparent in the +N ones in which the phloem flanking the root primordia is disintegrating. Since the pith is not highly active, all of the innermost root primordia and many of the meristematic areas in the pith are lacking.

UNWOUNDED INTERNODES.—The unwounded internodes of +N and −N plants reacted relatively as did the decapitated ones, with no pith development occurring in either case. The root primordia of the −N plants could rarely be induced to grow out, even when covered with moist sand. Figure 1*B* shows a +N plant which had been covered by moist sand at the place of treatment so that the root primordia lengthened and became a functional part of the root system.

Discussion

The reaction of the cabbage plant to applications of the hormone is very similar to that of bean as described by KRAUS, BROWN, and HAMNER (9). Cabbage when untreated produces no mass of callus tissue over the cut surface. In this respect it is unlike the bean but similar to the tomato (2) and *Iresine* (8).

The epidermis and cortex of cabbage respond similarly to those of bean, tomato, *Iresine*, and *Mirabilis*. Endodermis of cabbage reacts in a manner similar to that of tomato and *Mirabilis*, being less active than that of bean and more so than that of *Iresine*. Pericycle

of cabbage is inactive, resembling bean and tomato in this respect. The vascular tissues differ in their responsiveness from those of *Mirabilis*, the latter being relatively unresponsive while those of the other three are sensitive to applications.

Adventitious root production in cabbage closely parallels that of bean, in that it involves mainly the rays plus proliferated phloem, and does not involve pericycle as do *Iresine* and *Mirabilis*. Cabbage pith is very active and produces large numbers of irregular vascular strands which in later stages extend into the center of the stem; this reaction is similar to that of bean, and differs from *Mirabilis* in that its activity is initiated very early.

Inhibition or at least retardation of bud growth has been reported in cases where decapitated plants have been treated with growth promoting materials. In cabbage, as in *Nicotiana* (5) and *Lilium* (1), however, indoleacetic acid has been found to facilitate adventitious bud production from centers which were not meristematic previous to the treatment. In cabbage, although not in the other two plants, the chemical treatment merely induces the internal conditions requisite for expression of a capacity which normally rarely comes to expression. Just what determines which ones of the plants will produce shoots, however, is not known. Apparently each plant has a certain capacity for reaction, and this may be expressed in either root or shoot production or both. If shoots are produced, then the number of adventitious roots is greatly reduced.

It has been repeatedly noted by various workers that older plants or older regions of plants do not respond to applications of indoleacetic acid so rapidly nor to so great an extent as do young ones. The rate of this response in cabbage has already been noted. This is undoubtedly due to the state of maturity of the cells involved. Highly vegetative or young plants have relatively less differentiation of tissue and therefore a lesser maturity of cells than have feebly vegetative or older ones (10).

In general, the tendencies of the applications of the indoleacetic acid and the lack of nitrogen are to produce opposite effects on the cells. The former tends to cause a delay of maturity or, if the cell is already matured, first a resumption by it of that capacity for

division which is characteristic of immature cells (dedifferentiation), and then division. The lack of nitrogen, on the other hand, tends to hasten maturity of the cells, which sharply limits their capacity for division. Since most of the cells of the stems to which applications were made responded by cell enlargement or cell division or both, this resulted in a lessening of the effect of the chemical agent on the −N plants. This was expressed in fewer and slower cell divisions and a hastening of maturation after cell division, and therefore in a more limited amount of tissue involvement and in a lesser growth of new organs produced, such as root primordia. The lack of nitrogen hastened the maturity so greatly that the stimulated cells often divided only once or twice before maturing. This was particularly noticeable in the cortical cells. Many of those which were stimulated to divide matured into heavy walled fibers at once, resulting in many small scattered groups of those cells in the usually parenchymatous tissue. Thus in the −N plants, even though the hormone was able to overcome to a certain extent the tendency of the lack of nitrogen to hasten maturation and to prevent dedifferentiation of cells, yet the effect of the latter condition was marked.

The cells of the highly vegetative plants, most of which were in a relatively less matured state and had an ample food supply, were able to respond more readily and to a greater degree through continued divisions of their derivatives.

From a study of cellular reactions of various tissues of any one plant under different conditions, and from a comparison of the reaction of tissues of that plant with the same tissues of other plants, it is evident that, although indoleacetic acid may be the particular incitant in each case, yet it is not more than one factor in a causal complex. Reactions of the plant to it are dependent upon the hereditary constitution of the plant and the environmental factors affecting it, together with its past experience and the correlative effects of one part upon another part (11).

The fact that the unwounded plants did not react to so great an extent as the decapitated ones may be ascribed to the difficulty of penetration through the heavily cuticularized walls of the epidermis.

Summary

1. Applications of a 3 per cent mixture of indole(3)acetic acid in lanolin were made to decapitated stems and to unwounded internodes of cabbage plants grown in ordinary greenhouse soil. Similar treatment was given to plants half of which were grown under conditions of high nitrogen nutrition, the other half with a depletion of nitrogen.

2. Observations were made over a period of several weeks. The decapitated, soil-grown plants responded grossly by the production of masses of callus and root primordia.

3. All tissues of the stem responded to some extent to the treatment. The most generally responsive tissues were those of the phloem, rays, and pith. Cambium, cortex, endodermis, and xylem were moderately stimulated, while the epidermis and pericycle reacted weakly.

4. Adventitious roots were developed from ray and phloem tissue. Some internal roots were produced from pith cells adjacent to the primary xylem.

5. Near the surface of application confused mounds of callus tissue derived mainly from phloem and pith produced irregular groups of meristematic tissues in which great numbers of tracheids matured.

6. About one-third of the plants produced viable shoots, either directly from the top of the callus or laterally at about the level of adventitious root production. These lateral shoots were initiated by cell divisions either in the central or innermost cell layers of the cortex or in the rays, and always established organic union with vascular tissue of the main stem.

7. Stems treated laterally produced sunken areas through which in later stages the root primordia protruded. No mounds of callus were produced. No activity was apparent in the pith, but all other tissues reacted proportionally the same though to a less extent than in decapitated plants.

8. The reaction was slower in the $-N$ plants than in the $+N$

ones. They produced a much smaller callus, through which the root primordia rarely protruded.

9. The same tissues were involved in reactions of both +N and −N plants, although to a lesser extent in the latter. The pith and cortex of the −N plants were less active than in the +N ones, most divisions being confined to the rays, phloem, and endodermis. These, although the most active tissues in the −N stems, were still not nearly so reactive as those in the +N ones. The small amount of callus produced was less confused and showed greater vascularization than did that of the +N plants. Maturation of many cells, formerly parenchymatous, into fibers was characteristic.

10. Since the entire vascular cylinder was very unequally activated in the −N plants, far fewer root primordia were produced. These rarely grew out far enough to penetrate through cortex and epidermis.

The writer is indebted to the members of the botany department of the University of Chicago for helpful suggestions made during the course of this study.

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PITS IN THE HAPTERES OF NEREOCYSTIS

HARRIET W. ENGLERTH

(WITH FIVE FIGURES)

The presence of "sieve tubes" in the brown algae is well known; they have at least a superficial resemblance to sieve tubes of vascular plants, and occur in the center of the stipe. Surrounding them are cells which have conspicuous pits in their walls. It is with the nature of these pits that this paper is concerned.

Pitted cells were first reported by WILL (4) in *Macrocystis luxuriens*. The pits were in the lateral walls of the cells of the stipe. WILLE (5, 6), working on *Laminaria digitata*, *L. clusteni*, *L. saccharina*, and *Chorda filum*, found pits on the tangential walls of the cells and a few through the cross walls. They were closed by membranes. He called them macropores, while small holes which he thought he saw through the membranes he called micropores. ROSENTHAL (3) reported pits through the end walls of the cells of *Fucus vesiculosus*. HANSTEEN (1) found that the outer cells of *Fucus serratus* had usually only one pit between each two cells, but the outer cells of *Pelvetia* and *Sargassum* had more than one.

The plants of *Nereocystis* were gathered in Puget Sound at various times from October to April. The hapteres and the part of the stipe immediately above them were used. Small segments 1 cm. or less in length were cut and fixed in the following fluid: 100 ml. sea water, 1 gm. chromic acid, 5 ml. glacial acetic acid, and 1 drop hydrochloric acid. The pieces were subsequently dehydrated and imbedded in paraffin. Other pieces were cut freehand or with the freezing microtome. The use of alcohol and xylene apparently made the tissues hard and brittle. Dehydration and clearing with butanol by the method described by LANG (2) proved more satisfactory. Cutting fresh material either with pith or by freezing also gave good results. Both cross and longitudinal sections were made. Almost any general biological stain serves to demonstrate the pits in the walls, but it is more difficult to stain the delicate membrane which

extends across the pit. Of a large variety of stains tried, safranin, Bismark brown, and ruthenium red were most useful. A 0.25 per cent aqueous solution of safranin was used, 0.5 per cent solution of Bismark brown in 70 per cent alcohol, and 0.1 per cent slightly ammoniacal solution of ruthenium red.

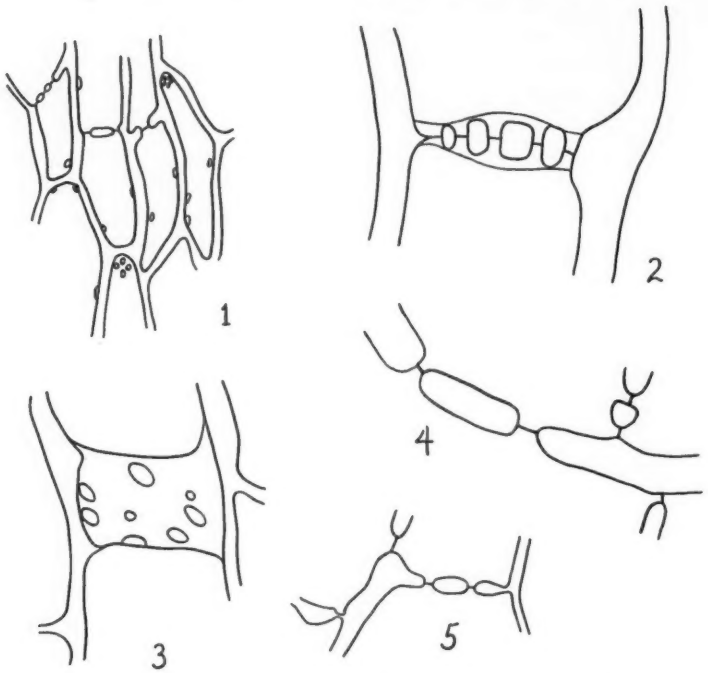
The membrane across the pit is so delicate that it easily escapes observation, especially after dehydration, which causes a marked shrinkage of the tissues. The larger pieces could be seen at times to shrink as much as 10 per cent. Soaking the cut sections in 1 per cent acetic acid counteracted this shrinking by causing a slight swelling in the walls and membranes. The sections could afterward be stained, dehydrated, and mounted in balsam without loss of detail.

Where the stalk ends and the hapteres begin, the "sieve tubes" are intermingled with the elongated cells. Some of the "sieve tubes" lie parallel with the axis of the stipe and abut on the elongated cells of the hapteres. These elongated cells (fig. 1) form the central region of each haptere. The outer cells of both the stipe and the haptere are smaller isodiametric cells.

The pits occur in practically all cells of the stipe outside of the "sieve tubes," and in all cells of the hapteres. Pits are most frequent on the tangential walls, but occur also on the radial and end walls. Each elongated cell of the stipe or center of the haptere has a circle of pits on the end wall, so that it resembles a true sieve tube except for the membranes across the pits. The smaller isodiametric cells of the stipe have fewer pits than the corresponding cells of the hapteres. A few pits were found in the inner walls of the epidermal cells.

The pits when seen in surface view appear to be holes through the walls (fig. 1), but when seen in profile (figs. 2, 4, 5) it is evident that each pit is crossed by a membrane. The cell walls of the hapteres are much thinner than those of the stipe, and the pits are smaller. Because of this any shrinkage makes it more difficult to see the pits. If the material has not been swelled by soaking in acetic acid, or if xylene was used for clearing, the walls may often be twisted, broken, or so thin that the membranes are not visible. From some of the pits the membrane (fig. 3) was torn away so that a hole resulted, but remnants of the membrane could be seen around

the edges. Many heavy stains such as anilin blue, or even safranin if destained too much, will not stain the membrane, and the pit appears as a hole, the colorless membrane being scarcely visible. Often sections are not cut exactly across the pit and the resulting appearance is that of a hole which does not extend quite through the wall.



FIGS. 1–5.—Fig. 1, elongated cells of haptere in radial section; ×200. Figs. 2–5, portions of walls of elongated cells of hapteres in radial section, showing pits; fig. 5 from distal region, others from base of haptere. Drawings made with camera lucida. ×750.

When examining the pit itself there often appear to be little nodules of some sort on the membrane, but on examination of many pits in both surface and profile, these nodules seem to be small particles of extraneous material gathered at each side of the pit. These, seen in surface view, may be what WILLE called micropores. The membranes of some of the pits seem to be slightly thicker in the middle.

The membrane through the pit distinguishes the pitted cells from the "sieve tubes," which are pierced by actual holes as far as is known at present.

This work was begun by the writer while at the University of Washington, and completed at Reed College. It was carried on under the direction of Dr. T. C. FRYE, University of Washington. Valuable aid and suggestions were also given by Dr. H. W. RICKETT of the University of Missouri.

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PHOTOPERIODIC PERCEPTION IN BILOXI SOY BEANS

H. A. BORTHWICK[1] AND M. W. PARKER[2]

(WITH ONE FIGURE)

Introduction

During recent years several investigators have concluded that flower primordia are initiated in photoperiodically sensitive plants in response to a stimulus received by the leaves and transmitted to the growing point (2, 7, 9). Many plants have been included in these various experiments, and some of the results have been summarized recently by CAJLACHJAN (3). Indirect evidence has been presented to show that a hormone "florigen" is responsible for flowering in photoperiodically sensitive plants (3). Data on many of these experiments are based entirely on the production of macroscopically visible flower buds or flowers. This practice seems open to criticism. There is some evidence (4) to indicate that flower primordia are sometimes initiated under certain environmental conditions that are not favorable to their further development into visible flowers. If this is true, it seems possible that in many cases flower primordia may be already present when photoperiodic experiments are begun. A critical morphological examination of representative plants should therefore be made before starting, and during the progress of, any photoperiodic experiments.

In the present investigation the influence of certain environmental factors on the initiation of flower primordia in the Biloxi soy bean was determined.

Observations

FLOWERING HABIT IN SOY BEAN

In previous work (1) attention has been called to the fact that the first visible flower primordia on soy bean plants appear in the axils of the fourth or fifth leaf from the apex of the main axis, and in

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corresponding positions on certain side branches. These first formed flower primordia occur on much shortened branches that arise in the axils of embryonic compound leaves.

It was found that when the treatment with short photoperiods is of only a few days' duration flower primordia are produced in a few axillary buds near the terminal, but no apparent change occurs in the terminal meristem itself. It continues to form primordia of compound leaves, in the axils of which the differentiating buds produce structures that are entirely vegetative, as was the case in the buds formed prior to the application of short photoperiods. If the treatment with short photoperiods is continued for a longer time, however, the growing point of the main axis stops forming primordia of compound leaves and forms instead primordia of simple bracteal leaves in the axil of each of which a single flower arises. This activity is the beginning of the formation of a terminal inflorescence. After a few of these bracteal leaves and flowers are produced, the growing point of the main axis ceases to elongate or to give rise to further lateral members.

After a soy bean plant has been subjected to a sufficient number of short photoperiods to cause the growing point of the main axis to begin initiating bracteal leaf primordia, it is difficult to cause the plant to resume the differentiation of purely vegetative structures at its apex by lengthening the photoperiod beyond the critical. If bracteal leaves have not yet appeared it is possible to prevent their differentiation by returning the plants to photoperiods longer than the critical.

In the following experiments induction periods have been made short so that their influence would not involve the meristem at the terminal of the main axis. Under these conditions different external environments cause differences in the number of axillary buds showing a photoperiodic response; thus a quantitative measure of the effectiveness of the treatment is available.

Intensity of supplemented light which prevents induction of flower primordia

It is recognized that artificial light of very low intensity used to prolong the photoperiod beyond the critical is effective in preventing

flowering of short day plants and in causing flowering of long day plants. The work of WITHROW (10), however, shows that the actual threshold values vary considerably in different plants. It therefore seemed desirable to obtain rather accurate data in this regard with the Biloxi soy bean.

Plants were grown on photoperiods of 16 hours, consisting of approximately 14 hours of natural light and 2 hours of Mazda, until they had three expanded compound leaves. At this time the plants were placed, for a period of 4 days, on photoperiods of 8 hours natural light plus 8 hours of Mazda light, the latter being varied in intensity from 0.6 to 100 foot candles. The controls for the experiment received only the 8 hours of natural light. At the conclusion of the 4 day treatment, all plants were returned to the original light conditions under which they were grown.

It was found when the plants were dissected 2 weeks later that initiation of flower primordia had occurred in the controls but in none of the others, indicating that 0.6 foot candle under these conditions was above the minimum necessary to prevent initiation of flower buds. Consequently, to determine the minimum another experiment was set up in which all the conditions were the same as in the first except that the intensity of supplemental light ranged from 5 to 0.01 foot candles. The results of this experiment (table 1) show that the intensities of supplementary light necessary to prevent induction lie between 1 and 0.5 foot candles. This result agrees with the first experiment in which 0.6 foot candle prevented initiation, and is also within the range found by WITHROW (10) for other plants.

It should be emphasized that these data relating to weak intensity are applicable only when low intensity light is used to extend a photoperiod of natural light beyond a critical day length.

INTENSITY OF LIGHT NECESSARY TO CAUSE INDUCTION

If induction is to be brought about by short photoperiods consisting entirely of artificial light, the intensity must be much greater than that used in lengthening photoperiods of natural light to prevent induction. Both Mazda and "sunshine" carbon arc lights were used in these experiments. Biloxi soy beans that had been growing in the greenhouse on a 16 hour photoperiod were transferred to

rooms where the temperature was held at 70° F. In one room the plants were subjected to intensities of 10, 40, and 100 foot candles of Mazda light; in the other to intensities of approximately 15, 150, and 1500 foot candles of light from a carbon arc.

The intensities of Mazda light were constant. The average intensity of light from the carbon arc was approximately constant, although there were continuous small fluctuations of very short duration. Measurements with a Weston foot candle meter showed that the intensity of light at the leaf surface of the plants subjected to

TABLE 1

EFFECT OF LOW INTENSITY MAZDA LIGHT WHEN USED TO SUPPLEMENT AN 8 HOUR DAY OF NATURAL DAYLIGHT

LIGHT INTENSITY USED FOR 8 HOURS TO SUPPLEMENT 8 HOURS OF NATURAL LIGHT (FOOT CANDLES)	TOTAL NODES ON MAIN AXES OF 10 PLANTS	NUMBER OF PLANTS WITH FLOWER PRIMORDIA	TOTAL BUDS ON MAIN AXES OF 10 PLANTS CONTAINING FLOWER PRIMORDIA
5	187	0	None
1	189	0	None
0.5	190	2	3
0.1	188	8	58
0.05	190	10	81
0.01 to 0.02	190	10	88
0.0 (controls)	188	10	102

approximately 150 foot candles did not go as low as 100 during these abrupt fluctuations. Likewise the high intensity did not go below 1000 nor the low intensity below 10. At each intensity plants were subjected to photoperiods of 8 and 16 hours, the latter serving as controls.

The plants were dissected after 5 days of treatment. Flower primordia were not initiated at any of the intensities produced by Mazda light. With the sunshine carbon arc light no flower primordia were present on the plants receiving 15 foot candles. At 150 foot candles one plant out of six had flower primordia in very early stages of development. Abundant flower buds were present on all plants that received 1500 foot candles. None of the controls in either type of light had flower primordia. These data indicate that the intensity

of light at the leaf surface must be above 100 foot candles during 8 hour photoperiods if flower primordia are to be initiated.

PHOTOPERIODIC RESPONSE WHEN TWO BRANCHED PLANTS ARE GIVEN DIFFERENTIAL PHOTOPERIODS

In some of their early studies of photoperiodism, GARNER and ALLARD (5) found on several varieties of plants that the effects of treatment were definitely localized. For example, in short day plants the parts subjected to photoperiods longer than the critical day length remained vegetative while the parts given photoperiods shorter than the critical produced flowers. Since the production of macroscopically visible buds or open flowers was used as the basis of observation, however, it is not certain that flower primordia may not have been initiated on the parts subjected to long photoperiod. While it may seem improbable, it is at least conceivable that flower buds might be initiated under such conditions and still fail to reach macroscopic size. In our work with Biloxi soy beans it has repeatedly been found that flower buds initiated by a short induction period develop very slowly when the plants are returned to long photoperiod, and in many cases never reach macroscopic size. It has also been found in our work that soy bean plants many months old initiate flower primordia abundantly, even though they have been grown on long photoperiod continuously. The development of these into macroscopically visible buds, however, rarely occurs if the long photoperiod is maintained. As a result the plants appear vegetative, and only by careful microscopic dissection is the true condition revealed. Experiments were accordingly set up to determine whether or not initiation of flower primordia is as strictly localized as is the production of visible buds and flowers.

For this work plants having two branches of nearly equal size were used. These were obtained by decapitating young plants just below the first compound leaf. The buds in the axils of the two primary leaves then expanded. The cotyledonary buds below usually failed to start growth; if they did they were removed. These plants were kept on a 16 hour photoperiod from the time they emerged from the soil until they were used in the experiments.

With plants of this type it was possible to apply short photoperiods to one branch while maintaining the other on long photoperiod.

In this work with two branched plants, the branch placed on 8 hour photoperiod is referred to as the donor and the one on 16 hour photoperiod as the receptor, in accordance with the terminology suggested by HAMNER and BONNER (**6**). After the donor branches had received five short photoperiods the differential treatments were discontinued and both branches were continued on photoperiods of 16 hours. On half of the plants used the receptor branches were defoliated, just prior to the beginning of the differential light treatments, leaving only the very small leaves at the terminal. The largest of these had not begun to expand at the beginning of the experiment, and during the 5 days of photoperiodic treatment any young leaves that showed a tendency to expand were removed. After the conclusion of the 5 days no further leaves were removed from the receptors.

At the beginning of the experiment the donor and receptor branches were approximately equal in size. Dissection of similar plants on the day the donors received their first short photoperiod showed that there was an average of thirteen nodes to each branch. The individual deviations from this mean were not more than one node.

At the conclusion of the experiment every bud was dissected on both branches of all the plants. The data from these dissections (table 2) show that initiation of flower buds occurred on receptor branches only when they were defoliated. Flower primordia on these receptors were not so abundant, however, as they were on the donors. This may be accounted for by the fact that the total number of nodes differentiated on defoliated receptors was reduced by defoliation.

These results show that initiation of flower buds was localized on donors if all the leaves were present on the receptors. Initiation was not localized, however, if all the leaves were removed from the receptors. Initiation of flower buds on receptors was therefore prevented by the presence of leaves.

Localization of induction reactions

The purpose of the following experiments was to differentiate between the action of various photoperiods when applied separately to the leaves and to the terminals of soy bean plants.

In order to give localized photoperiods to different parts of a plant, black cloth bags, made of double thickness black sateen, were employed. In direct sunlight of 10,000 foot candles they did not transmit sufficient light to activate a Weston foot candle meter which was sensitive to 0.01 foot candle. These bags were placed around a leaf or a terminal bud and fastened securely at the bottom;

TABLE 2

EFFECT OF PRESENCE OF LEAVES UPON FLOWER BUD INITIATION

No. of plants used	Treatment of branches	Total nodes in main axes of branches	Total buds on main axes of branches containing flower primordia
10	Donor not defoliated	168	37
	Receptor not defoliated	165	0
10	Donor not defoliated	172	40
	Receptor defoliated	158	15

the weight of the bag was supported by means of bamboo stakes. A typical setup is shown in figure 1.

The plants were grown under long photoperiod until they were just beginning to expand their third compound leaf. This leaf and the two primary leaves were removed to facilitate the procedure of applying localized photoperiods and to restrict the application of these to the two fully expanded compound leaves which still remained on the plant. The fact that the third compound leaf which was in the process of expansion was typical of neither the fully expanded leaves below it nor the unexpanded ones above constituted an additional reason for its removal. Dissection of representative plants at the beginning of treatment showed approximately fourteen nodes on the main axis, with no flower primordia present in any bud.

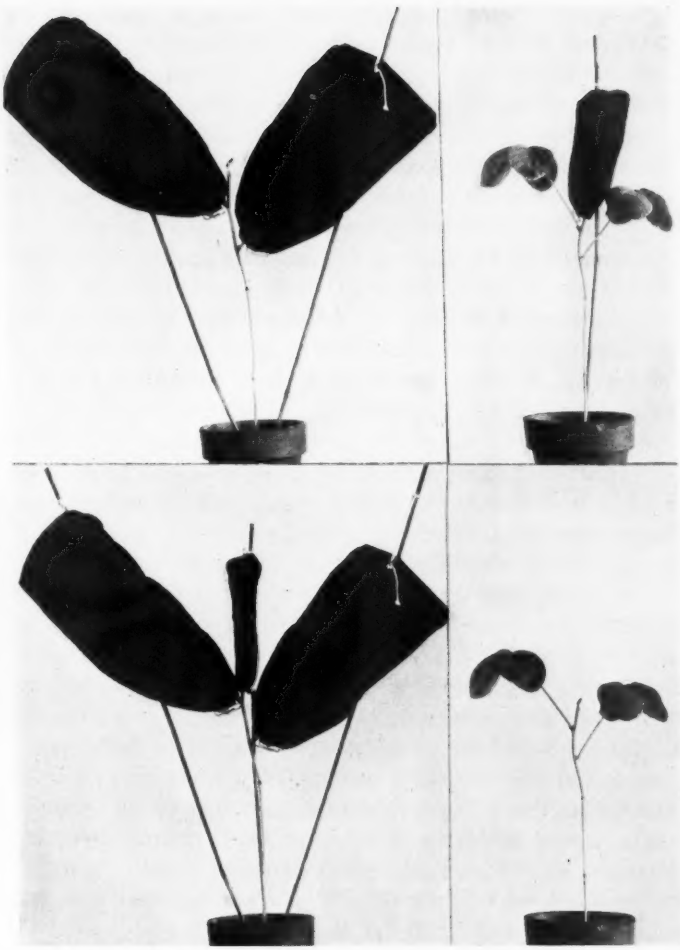

FIG. 1.—Size of plant and method of bagging used in experiments on localization of photoperiodic perception in Biloxi soy beans.

The terminal buds on three series of plants received a 16 hour photoperiod, an 8 hour photoperiod, and no photoperiod, respectively. By no photoperiod is meant that the parts concerned were kept in the dark continuously during the entire period of induction. Each series contained three lots of six plants. In one lot the leaves received a 16 hour photoperiod, in another an 8 hour photoperiod, and the remaining lot received no photoperiod. Thus photoperiods of different lengths were applied to leaves and terminal buds in various combinations. Controls were run with these lots to compare the effectiveness of black bags with large dark chambers, and also to demonstrate any effect caused directly by the removal of leaves. After three photoperiods the localized bagging was discontinued and all the plants, including the controls, were returned to a 16 hour photoperiod.

Microscopic examination was made of each node of all the plants in the experiment 18 days after their return to a long photoperiod. The controls showed that when whole plants were kept in continuous darkness for 3 days, whether in black bags or in a dark room, no flower primordia were formed. When plants were bagged from 4 P.M. to 8 A.M., however, flower buds were formed as abundantly as when the plants were kept in the darkroom during the same hours. Other controls showed that defoliation has no direct effect on initiation of flower primordia. Plants defoliated as just described and subjected to 8 hour photoperiods by means of cloth bags produced as many flower primordia as plants not defoliated but similarly bagged.

Comparison of lots in which photoperiods were applied locally to leaves and to terminals showed that initiation of flower primordia occurs only when the leaves are subjected to an 8 hour photoperiod. In all cases, when 8 hour photoperiods were applied to the leaves, induction occurred regardless of the photoperiod applied to the terminals. This reaction was not caused by the dark period alone, since no induction occurred when the leaves remained in complete darkness for the duration of the induction period and were then returned to long photoperiods.

In Biloxi soy beans the leaves are necessary to produce the stimulus causing initiation of flower primordia. This stimulus moves read-

ily in the plant, and under the conditions of this experiment causes a response in the buds near the terminal growing point, regardless of the photoperiod being applied to that region. The stimulus received during a 3 day induction period was sufficient to cause initiation of flower primordia in buds that had reached a certain stage of development. Other buds, some older and some younger, were not influenced by this short induction period and remained vegetative.

Since an 8 hour photoperiod on the leaves causes initiation, the question arises as to whether the photoperiodic response would still occur if individual leaves of the same plant received their short photoperiods at different hours of the day. Investigation of this point was made with plants that were just expanding their third compound leaf. This leaf, the two cotyledons, and the two primary leaves were removed, leaving only two compound leaves on the plant. These two leaves, which were located at the third and fourth nodes, were subjected to 8 hour photoperiods, one leaf receiving its photoperiod from 4 A.M. to noon and the other from noon until 8 P.M. Each leaf received a dark period of 16 hours following its photoperiod. In half of the plants the upper of the two leaves received its photoperiod in the morning, and in the other half in the afternoon. Controls were run in which one of the two leaves was bagged continuously and the other was subjected to 8 hour photoperiods. The upper leaf was given the 8 hour photoperiod in one lot of these and the lower in the other. In a third group of controls both leaves received 8 hour photoperiods simultaneously; in a fourth, both were subjected to 16 hour photoperiods. Treatment of the experimental plants and the controls was continued for 4 days, after which they were returned to 16 hour photoperiods.

In this experiment the combined photoperiods amount to 16 hours, which is above the critical for the Biloxi soy bean. Considered from this point of view the buds in the terminal might be expected to remain vegetative. On the other hand each leaf received an 8 hour photoperiod and a 16 hour dark period. These conditions should result in rapid initiation of flower buds, provided each leaf acts independently.

In the experimental plants one leaf of which received its 8 hour photoperiod in the morning and the other in the afternoon, flower primordia were present in the buds near the terminal regardless of which leaf received its photoperiod first. In the controls it was found that when both leaves were simultaneously subjected to 16 hour photoperiods no flowers were formed. If both were subjected simultaneously to 8 hour photoperiods, however, flower primordia were initiated. They were also initiated when either of the two leaves was kept continuously in the dark while the other was subjected to 8 hour photoperiods. These results, although preliminary, seem to indicate that flower bud formation is determined by the photoperiod given the individual leaves and not by the combined length of the photoperiods given alternately to the two leaves.

Discussion

When attempting to explain the mechanism of photoperiodism it is essential to know the morphological condition of the plants before and after differential light treatment. Induction of flower primordia may have already occurred but owing to the subsequent environment these primordia may not continue to develop. With some plants this fact would be more important than with others, since EGUCHI (4) has shown that in some cases environments suitable for induction are also suitable for development, while in others the environment associated with induction is not suitable for continued development of the primordia. In either case only plants of known morphological condition should be used for physiological studies of photoperiodism.

In the work of LOEHWING (8) with Ito San soy beans no dissection data are given, but one would infer from the fact that flowers began to appear 10 days after the start of short day treatment that their primordia must have been well established before the treatments were started. Biloxi soy beans require about 3 weeks from the beginning of induction to the opening of first flowers. Ito San soy beans grown at Beltsville on 14 hour photoperiod for 22 days initiated abundant flower primordia. Other Ito San plants grown con-

tinuously on an 18 hour photoperiod also formed flower primordia. There was no microscopic evidence of these until the plants were nearly 4 weeks old.

It is entirely possible that Ito San plants grown at a different location and at a different time of year may show a different morphological response. The results indicate, however, the extreme importance of careful morphological examination of representative plants at the beginning of all photoperiodic experiments as well as at their termination.

The results here presented show that the leaves of Biloxi soy beans are the organs of photoperiodic perception. This is in accord with the findings of other investigators with various plants. Initiation of flower buds does not occur in soy beans if they are subjected to darkness during the entire experimental period. Whether the critical reaction that leads to flower bud formation is one that occurs in light or in dark is not clear from the data thus far obtained, for it has not yet been possible with this plant to reduce the induction period to less than two short photoperiods and two long dark periods.

Induction does not occur when 8 hour photoperiods of natural daylight are extended to 16 hours by adding 8 more hours of Mazda light of intensity as low as 0.6 foot candle. This additional 8 hours of low intensity may be regarded as effective either in continuing a photochemical reaction or in preventing a reaction that requires almost complete darkness. Since it has been shown, however, that the intensity of light during the 8 hour photoperiod must be greater than 100 foot candles if initiation is to occur, it seems that in the soy bean the response is in some way influenced by conditions occurring during the light period.

The data obtained show that a short photoperiod results in a reaction in the leaves, the effects of which when transmitted to certain buds will regulate their differentiation. Several investigators have proposed that flowering in photoperiodically sensitive plants is induced by the action of a flower forming hormone. Whether the interpretation is correct or whether the responses secured are due to other causes remains to be determined.

Summary

1. When whole plants of Biloxi soy beans are subjected to 8 hour photoperiods, initiation occurs if the intensity of light during the photoperiod is above 100 foot candles. Below 100 foot candles no flower primordia are initiated.

2. When plants are given an 8 hour photoperiod of natural light supplemented by 8 hours of Mazda light, initiation occurs if the intensity of the supplemental light is below 0.5 foot candle, but does not occur if the intensity is above 0.5 foot candle.

3. The stimulus that causes initiation of flower primordia at the growing points arises in the leaves and moves to the growing plants.

4. Flower primordia may be initiated at growing points that are kept, either in complete darkness or on photoperiods above the critical, provided the leaves are kept on short photoperiods.

5. Photoperiods shorter than the critical, applied directly to growing points, have no effect on flower bud initiation. Control of initiation is exercised only through application of photoperiods of proper length to the leaves.

6. Whether the responses secured in these experiments are due to a flower forming hormone or to other causes remains to be determined.

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PHOTOPERIODISM IN RELATION TO HORMONES AS FACTORS IN FLORAL INITIATION AND DEVELOPMENT[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 496

KARL C. HAMNER AND JAMES BONNER

(WITH ELEVEN FIGURES)

I. Introduction

In 1925, GARNER and ALLARD (7) showed that, for some plants, the response to photoperiod is localized; that is, the separate portions of the plant directly subjected to the various photoperiods responded individually rather than there being a general systemic response of the entire plant. KNOTT (10) demonstrated in 1934 that the initial effect of photoperiod is received by the leaves in spinach, a long day plant. Subsequently, workers in Russia, CAJLACHJAN (2), MOSKOV (16), LUBIMENKO (13), and others, extended the work of KNOTT to other plants and confirmed his view that the leaves are the organs which receive the initial photoperiodic stimulus. Extensive studies of the movement of the effect within the plant and across graft unions have also been made. This work has recently been summarized by CAJLACHJAN (5).

Following the subjection of plants to photoperiods conducive to flowering, there seem to be produced certain effects which are capable of being transported within the plant, and across graft unions. The hypothesis has been advanced by CAJLACHJAN and others (3, 11, 12, 16) that a specific flower forming hormone is manufactured by leaves under certain photoperiods, and that this may move to various parts, resulting in floral initiation and often in subsequent development. SACHS (17) suggested that specific flower forming substances probably exist. His experiments also indicated that such a substance must be supplied from the leaves. It remains

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

to be established that the flowering response is actually controlled by a specific chemical substance.

It is desirable to bear in mind precisely what is meant by the term "flowering condition" of plants (**9**). This term may be divided into at least six stages: (1) induction of the flowering condition; (2) initiation of the flower primordia; (3) development of these primordia into macroscopic flowers; (4) development of the fruit; (5) gametic union; and (6) development of seeds. There may well be many hormones which influence the flowering of plants, and not only may each particular hormone be involved in a single stage of the process, but there may be many interactions as well. The present work is concerned mainly with a study of the conditions leading to the induction and initiation of flower primordia, and their possible subsequent development.

Xanthium pennsylvanicum, a cocklebur, was chosen as the principal experimental material. If grown continuously under ordinary greenhouse conditions at photoperiods in excess of 16 hours, it remains vegetative indefinitely. It is a short day plant, possesses a sharp critical period, and blooms at photoperiods shorter than 15 hours. Its floral primordia are readily recognizable under the dissecting microscope within 5 days after transfer of plants from long to short photoperiod. It possesses the advantage also that once floral primordia have been initiated under short photoperiods, they develop into flowers and fruits despite subsequent wide variations in environmental conditions. *Xanthium* grows rapidly, and if subjected to short photoperiods will flower in 14 days after emergence of the cotyledons from the soil.

II. Terminology and experimental methods

For the experiments reported here mature fruits of *Xanthium pennsylvanicum* were collected in November, and others which had passed the winter out of doors were collected in the vicinity of Chicago in early May. All were stored at room temperature in a dry place until used. The seeds were removed from the fruit, scratched slightly to rupture the seed coats, and after removal from the bur were planted within a few days in an open garden soil. Four seeds were sown in 4.5 inch pots, or 100 seeds in an ordinary greenhouse

flat. After the seedlings had attained a height of 6–10 inches, they were transplanted singly to 3.5 inch pots in order to obtain sturdy plants.

In certain experiments (section X) the seeds were planted in pure quartz sand contained in 2 gallon glazed crocks, and supplied on alternate days with 500 cc. of SHIVE's slightly modified R_2S_5 nutrient solution (18). On intervening days the plants were supplied with distilled water. Such accessory growth factors as were used were

FIG. 1.—Plants at stage of development used in most experiments. From left to right: defoliated at base, defoliated at tip, intact (not defoliated), and two branched.

added to the nutrient solution just prior to its application to the crock.

Excessively high temperatures or low humidities were minimized by ventilation of the greenhouse and frequent spraying of the walks and benches. The plants were well watered and grew vigorously throughout the experimental period. In most of the experiments (those labeled higher than X-13) the plants were 3–5 weeks old. The plants used in experiments labeled X-1 to X-13, however, were more than 6 weeks old when used, and in all experiments concerned with two branched plants, the plants were 4–6 weeks old (fig. 1). All plants were grown until used for experimentation on evenly lighted benches in the greenhouse. The natural daylight was supplemented with Mazda light of about 100 foot candles at the surface

of the leaves. These lights were burned from 7 P.M. until midnight in all experiments up to August 17, and from 6 P.M. until 2 A.M. after August 18. The photoperiod thus varied from 18.5 to 20.5 hours. Plants maintained continuously under these conditions of long photoperiod remained strictly vegetative. The term long photoperiod, as used in these experiments, means one in excess of 18.5 hours accompanied by a dark period of less than 5.5 hours. The term short photoperiod, as used in this work, refers to a photoperiod of 9 hours' duration with an accompanying dark period of 15 hours.

Great care was taken to maintain accurately the desired photoperiod. Lamps were checked daily, and plants were dissected periodically to make certain that they were in fact strictly vegetative. As a result of this checking, several thousand plants were discarded because of the accidental failure of the supplementary illumination for two nights. In every experiment also, control plants were dissected at the beginning and at the end of the experimental period. No experiment is reported in which control plants on long photoperiod or on 16 hour photoperiod were not strictly vegetative. With few exceptions, as noted in the tables, the plants subjected to a specific treatment exhibited a uniform response; that is, either all plants were strictly vegetative, or all plants possessed floral primordia or flowers at the same stage of development.

When it was desired to control accurately the photoperiod to which a group of plants was subjected, special trucks were used. During the day, in general for 9 hours, these trucks stood in an evenly lighted greenhouse. Each evening each truck was rolled into its light-tight compartment, where it remained until the following morning. In this way the plants received 9 hours of natural daylight, supplemented with artificial light of any desired duration from Mazda lamps controlled by time clocks. A series of smaller and simpler compartments were also used (fig. 2). Each evening the frames of these compartments were covered with black sateen cloth, two layers thick. These covers were removed each morning at 8 A.M. This set of seven compartments, each on a separate time clock, proved particularly useful for experiments in which it was desired to grow a few plants on each of a number of different photo-

periods; that is, for determination of critical period, for interruption of the dark period by short light periods, etc.

In the experiments in which vegetative plants were grown as close to the critical day length as possible, the 9 hours of natural daylight were supplemented by light from Mazda lamps for 7 hours, thus making a total of 16 hours, a photoperiod just in excess of the critical[2] (section IV). This supplementary light was given immediately after the 9 hours of daylight on certain trucks, on others it was given immediately before. These two types of 16 hour photoperiods

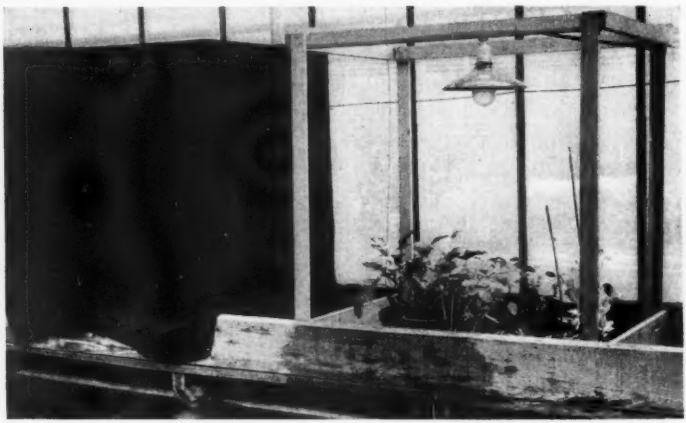

FIG. 2.—Type of small compartments used in experiments. The one at left has cover in place. Each controlled by separate time clock.

were used in the experiments of section IV. Plants which had been growing on a truck receiving supplementary light in the evening were removed during the day and placed on a truck receiving supplementary light in the early morning. These transferred plants then received a short photoperiod of 9 hours. The following dark period, however, was for them of 8 hours' duration. In this way the plants growing continuously on 16 hour photoperiods were given one short photoperiod without an accompanying long dark period.

[2] In some experiments, noted in the tables, a 15.5 hour day was used. In these experiments the controls were vegetative (15.5 hours was above the critical period).

A standard, relatively simple procedure was developed for treating one portion of a plant with short photoperiod and the rest of the plant with long photoperiod. Cardboard cans were painted inside with flat black and outside with aluminum paint. The covers of the cans were removed, slit, and provided with a central hole (fig. 3). The covers were then slipped in an inverted position on to the stem of the plant, the stem passing through the central hole. The radial slit was then sealed with adhesive tape and the cover fastened firmly with thumbtacks to a wooden frame, to which the pot was also wired (fig. 3). The body of the can could then be inverted over the plant and slipped into position in the cover. The light intensity inside such a can is less than 0.01 foot candle even in bright sunlight, and the temperature was never more than 2° F. above the temperature of the surrounding greenhouse air.

With this apparatus it was possible to obtain any combination of long and short photoperiod on different parts of the same plant. It was easy to assemble, and as many as 250 cans have been placed in position night and morning by two people, the entire process consuming about 30 minutes.

In section IV experiments are described in which plants were submitted to various night temperatures. These plants were kept in the greenhouse during their photoperiod, and then transported for the dark period to darkrooms or compartments maintained at the required temperatures. Each morning the plants were returned to the greenhouse.

In the experiment in which a temperature of 4° C. was maintained during the photoperiod, a room equipped with a cooling unit was used. This room was equipped with a carbon arc light, supplying about 900 foot candles at the leaf surface. A similar room kept at 21° C., illuminated with a carbon arc light for 10 hours per day and Mazda light for an additional 6 hours, was used for many of the experiments with cuttings as described in section X.

By a vegetative plant is meant a plant which does not possess either flowers or recognizable floral primordia, and which, if maintained continuously on long photoperiod, does not develop them. The possession of one or more flowers or floral primordia is con-

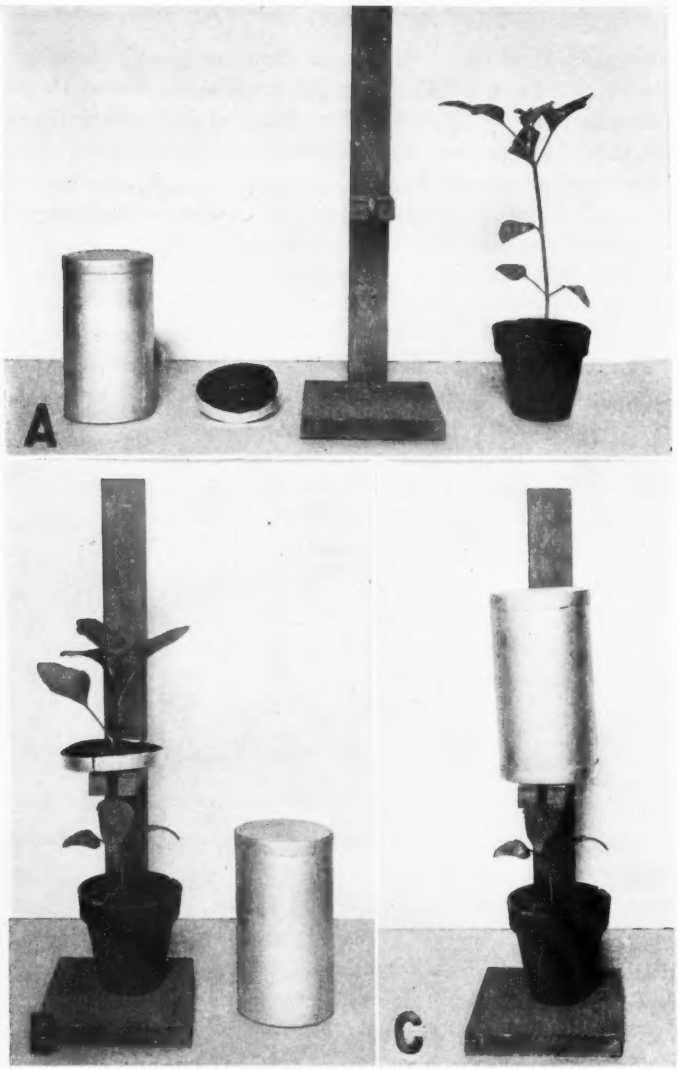

FIG. 3.—Apparatus used to subject whole or parts of plants to same or different photoperiods at same time. *A:* aluminum coated paper box and cover, wooden standard, and potted plant; *B:* plant and cover in place; *C:* box in place for darkening upper portion of plant while lower portion is still exposed to light.

sidered to represent the flowering condition. As a matter of routine, at least the terminal buds on all the plants of any experiment were dissected about 14 days after its start, in order to determine whether or not floral primordia were present. Figure 4 shows some of the stages of development recognizable upon dissection.

In cases where two large branches per plant were desired, the plants were severed at the first internode when the latter had attained a length of 0.5 to 1.0 cm., or approximately 15 days after planting the seed. As a result both cotyledonary buds expanded in many cases.

As will be discussed later, it has been found in this work that a portion of a plant exposed to short photoperiod produces an effect upon other portions of the plant not so exposed. In order to facilitate discussion, the portion of the plant on short photoperiod will be referred to as the "donor" portion, and other portions which initiate floral primordia as a result of substances received from this donor will be called "receptor" portions.

III. Evidence for a specific substance involved in floral initiation

A given plant response can definitely be linked with a given specific substance only when this substance has been isolated, chemically identified, and shown to be essential to a particular response. Experience with other plant hormones, such as auxins and vitamin B_1, has indicated that correlations within the plant are often to be attributed to chemical substances having the properties of hormones. Floral initiation might be conclusively attributed to the presence of a substance or substances if it could be shown that the effect could be linked to something which is diffusible, can pass out of one plant and back into the same or another plant, and there bring about initiation of floral primordia. Such a substance or substances might be considered as hormones. The results of the detailed experiments which follow give strong indication that hormones are involved in the initiation and development of floral primordia, flowers, and fruits by plants subjected to varying photoperiods and dark periods under certain environmental conditions.

When a *Xanthium* plant is subjected to short photoperiod, the

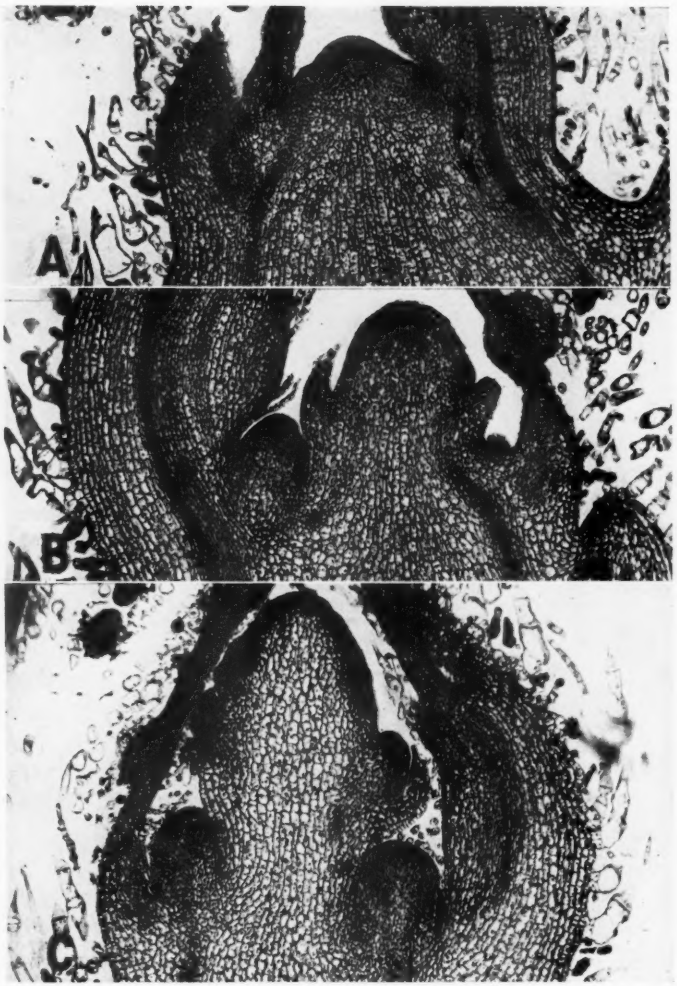

FIG. 4.—Median longitudinal sections of terminal buds of *Xanthium*. *A:* strictly vegetative; *B:* inflorescence primordium; *C:* later stage in development of inflorescence. Stages between *B* and *C* designated as inflorescence primordia in the tables; stages between *C* and macroscopic flowers designated as flower primordia.

initial stimulus to floral initiation is received by the leaves, as is shown by three types of experiments.

1. Sturdy vegetative plants were placed on short photoperiods. One lot of these was completely defoliated and the other left undefoliated. The leafy plants were found to have large flower buds after 11 days. The defoliated plants remained strictly vegetative for 3 weeks, after which the experiment was discontinued (table 5).

2. In other experiments a single fully expanded leaf of each plant was exposed to short photoperiod while the rest of the plant received long photoperiod. These experiments, summarized in table 1, show

TABLE 1*

FLORAL INITIATION AS RESULT OF EXPOSURE OF SINGLE LEAF TO SHORT PHOTOPERIOD

EXPERIMENT NO.	TREATMENT	DURATION OF TREATMENT (DAYS)	NO. OF PLANTS	CONDITION OF PLANT AT END OF TREATMENT
X-23 and X-41	One leaf on short photoperiod	18	32	mac fl & fr
	Untreated controls	18	6	veg

* In this and all subsequent tables the condition of the terminal bud at the time of dissection is indicated as follows:
veg: strictly vegetative.
infl pr: inflorescence primordia.
fl pr: flower primordia.
mac fl & fr: macroscopic flowers and fruits.
Unless otherwise stated, all plants which received a given treatment were in the same stage of floral development. In all tables the term untreated controls refers to those plants which were maintained on a long photoperiod at the side of the experimental plants, whenever the latter were exposed to long photoperiod, and serve to show whether or not supplementary illumination may have failed at any time during the experiment.

that one leaf exposed to short photoperiod may be sufficient to result in the initiation of flower buds and the development of macroscopic flowers, even though the remainder of the plant is growing on long photoperiod. An example of such a plant after the leaf had received 20 short days is shown in figure 5.

3. In an experiment of another kind two branched plants were used. One branch received short and the other long photoperiod. In one lot of plants the short day branch or donor was defoliated, and in the other it was left intact. In table 2 it may be seen that if, at the time it is subjected to short photoperiods, the donor branch pos-

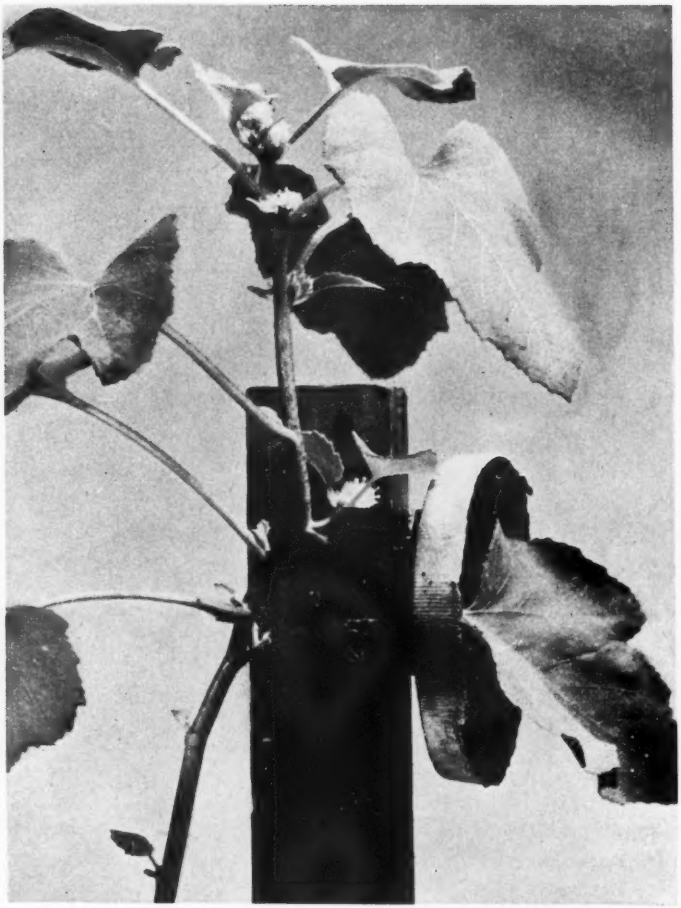

FIG. 5.—Development of flowers on a plant one leaf of which (shown at right of cover) has been exposed to 20 short photoperiods; remainder subjected to long photoperiods only.

sesses leaves, initiation of flower buds takes place on the donor and receptor branches. If such a short day branch had previously been defoliated, both branches would remain completely vegetative.

A number of experiments were performed in order to determine in what stage of development leaves are most effective in the perception of the photoperiodic stimulus. A group of vegetative plants was selected and divided into four lots. One lot was completely defoliated, one not defoliated in any way, one defoliated except for the young expanding leaves, and the fourth defoliated except for one fully expanded leaf. Of the last lot, the area of the expanded leaf on

TABLE 2

EFFECT OF DEFOLIATION OF BRANCH EXPOSED TO SHORT PHOTOPERIOD ON FLORAL INITIATION IN BRANCH EXPOSED TO LONG PHOTOPERIOD

Experiment no.	Treatment	No. of plants	Duration of treatment (days)	Condition of receptor branch
X-37	Donor branch defoliated	6	14	veg
	Donor branch leafy	6	14	mac fl & fr
	Untreated controls	6	14	veg

any given plant was reduced by cutting away part of it, leaving approximately 2–3 sq. cm., an area about equal to that of the young expanding leaves on other plants. All plants were subjected to short photoperiod, some plants of each lot receiving one short photoperiod (with an accompanying long dark period), other plants two, and so on. During the course of the treatment, as young leaves expanded, the plants were defoliated in order to maintain approximately the same leaf area on any given plant as was present at the start of the experiment (table 13).

Undefoliated control plants required but one short photoperiod (with an accompanying long dark period) for the subsequent initiation of floral primordia, while completely defoliated control plants remained vegetative even after ten short photoperiods. The plants which possessed a portion of one mature leaf initiated floral primor-

dia after receiving three short photoperiods. The plants having an equal area of young leaves, on the other hand, remained strictly vegetative even after seven short photoperiods. This experiment and others indicate that mature leaves are much more effective than are young leaves in the direct perception of the photoperiodic stimulus.

Since the initial photoperiodic stimulus is received by the leaves, the effects of this stimulus must be capable of transport within the plant to reach the bud or buds. A number of special experiments

TABLE 3

MOVEMENT OF STIMULUS TO FLORAL INITIATION WITHIN THE PLANT

EXPERIMENT NO.	TREATMENT	NO. OF PLANTS	DURATION OF TREATMENT (DAYS)	CONDITION IN PART OF PLANT EXPOSED TO LONG PHOTOPERIOD
X-2	Tip of plant on short photoperiod; base on long	12	17	fl pr
	Base of plant on short photoperiod; tip on long	12	17	fl pr
	Untreated controls	6	17	veg

concerning this movement have been performed. Localized portions of several plants were subjected to short photoperiod. On some several mature apical leaves were so treated; on others, mature basal leaves were used. The remainder of the plant in each case was kept on a 15.5 hour photoperiod. Other plants, not treated with short photoperiod, were kept on a 15.5 hour photoperiod as controls. Whether the top or the base alone was subjected to short photoperiod, flower primordia formed throughout the length of the plant. Controls remained vegetative (table 3). Thus the effect of short photoperiod moves both up and down the stem. This observation was confirmed in many other ways, perhaps the most convincing of which was the type of experiment in which two branched plants were used. One branch was placed under short

photoperiod, the other under either 16 hour or long photoperiod. Details of such experiments are given in tables 2, 11, 12, 15, and 16. In every case in which the short day and the long day branch were not defoliated in any way, it was found that the long day branch as well as the short day one initiated floral primordia and developed macroscopic flowers within 2 weeks. The stimulus for floral initiation must therefore be capable of moving down and out of the short day donor portion and up and into the long day receptor portion to the terminal bud of the latter.

That the upward movement of the stimulus probably does not depend upon a passive transport in the transpiration stream is indicated by experiments which were carried out in a nearly saturated atmosphere where transpiration would be expected to be low. Under these conditions also, leaves exposed to short photoperiod at the base of the plant resulted in the initiation of floral primordia at the tip.

The stimulus for the initiation of floral primordia is capable not only of movement within any given plant, but also from one plant to another. A considerable number of splice grafting experiments were carried out in the usual manner, a scion being splice grafted to a stock and the grafted plant placed in a chamber at high humidity and under the desired photoperiod. This method was found to be more difficult to carry out and to be of less wide applicability than the approach graft method, in which portions of the stems of two plants were shaved longitudinally to the cambium and the two shaved surfaces bound together with raffia (fig. 6). The leaves above the graft of one of the plants were then placed on short photoperiod and those of the other kept on long photoperiod. In each experiment two kinds of contacts were made. In the one the union was not interfered with in any way and the stock and scion united within 10 days. In the second, or "diffusion contact" type, a layer of lens paper was placed between the two members. Histological examination showed that the paper prevented the formation of a tissue connection for at least 14 days. In fact, in many cases the two members were found to fall apart as soon as the raffia binding was removed. Only soluble substances should be able to pass across such a diffusion contact.

FIG. 6.—Diffusion contact between two vegetative plants on June 28. Subsequent to that date, tip of plant shown above the cover was subjected to short photoperiod, the other defoliated and continued on long photoperiod. Both plants flowered and fruited. Small leaves on plant under long photoperiod developed after experiment was begun. Photograph taken July 26.

In the experiments recorded in table 4 both plants were initially vegetative. After the graft was made, one member was subjected to short photoperiod and the other to either the long or 16 hour photoperiods. The stimulus causing floral initiation apparently moved readily across an ordinary graft union, since the long day member of the graft developed floral primordia. This might be expected from the fact that the same stimulus moves so readily within any given

TABLE 4

PASSAGE OF STIMULUS FOR FLORAL INITIATION ACROSS DIFFUSION CONTACTS AND ACROSS GRAFT UNIONS. EXPERIMENTS X-6, X-12, X-13, X-17, X-34

TREATMENT	NO. OF DIFFUSION CONTACTS OR GRAFTS	DURATION OF TREATMENT	CONDITION OF RECEPTOR AT END OF TREATMENT
Approach graft.....	10	12–33 days	8 fl pr 2 veg
Diffusion contact.....	18	12–33 days	17 fl pr 1 veg
Untreated controls...	15 (plants)	12–33 days	veg

plant. The stimulus is also able to diffuse from one plant merely in close contact with another, indicating definitely that the stimulus is a substance or substances. This is, so far as the writers are aware, the first direct evidence which has been presented concerning this point.

IV. Floral initiation as controlled by length of dark period

It seems probable that the manufacture of the substance or substances responsible for the initiation of the flowering condition in *Xanthium* is not primarily a response to duration of the photoperiod, but rather a response to duration of the dark period.

In a preliminary experiment carried out in May, 1938, it was found that *Xanthium* plants grown continuously on photoperiods shorter than 15 hours invariably flowered, while plants grown continuously on photoperiods longer than 15.5 hours failed to initiate floral primordia. In a similar experiment carried out in August, 1938,

it was found that this critical photoperiod lay between 15.5 and 15.75 hours' daily illumination. Thus although the critical photoperiod varies somewhat with change of environmental factors, possibly in this case with changes of temperature, still it remained fairly constant during the experimental period. In order to decide whether the "critical day length" is determined primarily by the length of photoperiod or by the length of dark period, experiments were carried out using cycles of other than 24 hours' duration.

The critical photoperiod is as indicated of approximately 15.5 hours, with the critical dark period of approximately 8.5 hours. If the length of the daily illumination period is the determining factor,

TABLE 5

EFFECT OF COMPLETE DEFOLIATION UPON FLORAL INITIATION IN XANTHIUM PLANTS EXPOSED TO SHORT PHOTOPERIOD

EXPERIMENT NO.	TREATMENT	NO. OF PLANTS	DURATION OF TREATMENT (DAYS)	CONDITION OF PLANT AT END OF TREATMENT
X-1	Completely defoliated	6	21	veg
	Leafy control	6	21	mac fl & fr

then *Xanthium* plants should initiate floral primordia on any cycle having photoperiods of less than 15 hours, and should fail to flower on any cycle having photoperiods longer than 16 hours. If length of dark period is the determining factor the reverse should be found; plants should initiate floral primordia on any cycle having dark periods longer than 8.5 hours, and should fail to flower on any cycle having dark periods shorter than 8.5 hours, irrespective of shortness of the photoperiod. Since it has been determined that *Xanthium* initiates floral primordia and flowers profusely on a 24 hour cycle, consisting of an 8 hour photoperiod and a 16 hour dark period, two other cycles were chosen in which the ratio of photoperiod to dark period was also 1:2. Thus a 12 hour cycle, consisting of a 4 hour photoperiod and an 8 hour dark period, and a 48 hour cycle consisting of a 16 hour photoperiod and a 32 hour dark period, were used. Table 6 shows that plants grown on the 12 hour

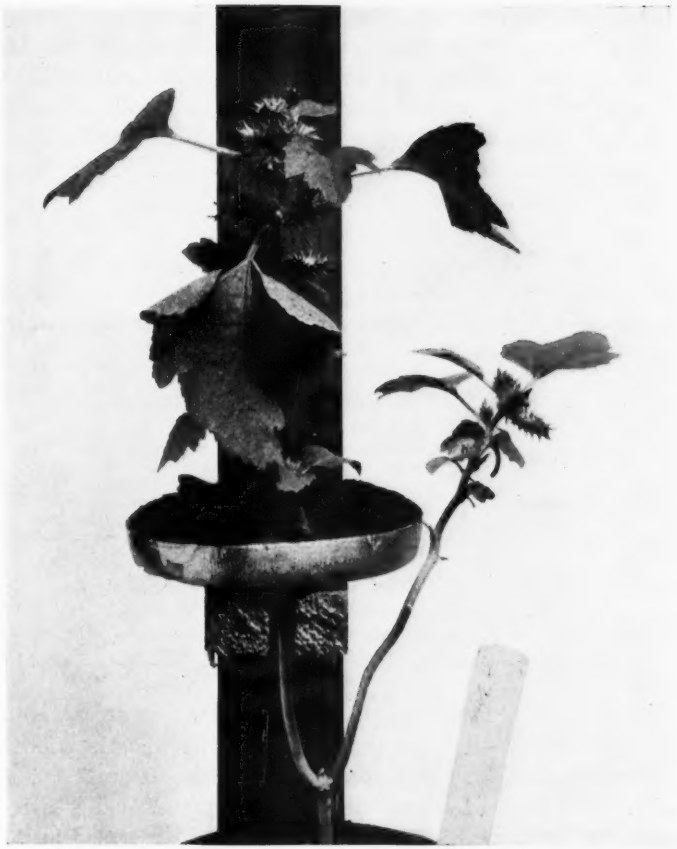

FIG. 7.—At the beginning of the experiment, the two branches of the plant were approximately equal in size, and vegetative. One (donor) was covered and received 33 subsequent short photoperiods; the other (receptor) had the older leaves at the base removed but was continued on long photoperiod. Both shoots fruited. Young leaves on receptor have not prevented initiation of flower primordia nor development of fruits (*see* fig. 8).

cycle with a short photoperiod and a short dark period remained vegetative, whereas the plants grown on a 48 hour cycle with a long photoperiod and a long dark period initiated floral primordia and flowered profusely. *Xanthium* initiates floral primordia when subjected to long dark periods even if the photoperiods are also long; it does not initiate floral primordia when subjected to short photoperiod if the dark periods are also short.

Another and more direct type of experiment supports the view that it is length of dark period which results in the initiation of floral primordia by *Xanthium*. Plants growing on the 16 hour photoperiod were given a single 40 hour dark period by substitution of a dark period for one 16 hour photoperiod. Similar plants were given a single short photoperiod of 9 hours, but with uninterrupted dark periods of 8 hours' duration. This was accomplished by the substitution of one 17 hour cycle for the usual 24 hour cycle (section II). On the basis of numerous repetitions of this experiment (table 7), it is evident that under certain conditions (see page 409) a single long dark period, unaccompanied by a short photoperiod, is sufficient to result in the subsequent initiation of floral primordia. One short photoperiod unaccompanied by a long dark period has been found to be completely without effect upon the initiation of floral primordia.

TABLE 6

EFFECT ON FLORAL INITIATION OF CYCLES OTHER THAN 24 HOURS
EXPERIMENT X-62

LENGTH OF CYCLE AND PHOTOPERIOD	NO. OF PLANTS	CONDITION OF PLANTS AFTER 15 DAYS
12 hour cycle { 4 hour photoperiod ..... 8 hour dark period	10	veg
48 hour cycle { 16 hour photoperiod ..... 32 hour dark period	10	mac fl & fr

If a process involved in the initiation of floral primordia by *Xanthium* takes place at night, it would seem probable that it is one which is adversely affected by light. That this is the case is demonstrated by the following experiment (table 8). Vegetative plants

were removed from long photoperiod and placed on a 9 hour photoperiod of daylight in the greenhouse. This was supplemented by 6 hours of artificial light to give a 15 hour photoperiod and a 9 hour dark period, upon which *Xanthium* flowers abundantly. If, however,

TABLE 7

EFFECT OF EXPOSURE TO ONE LONG DARK PERIOD OR TO ONE SHORT PHOTOPERIOD OR TO BOTH. EXPERIMENTS X-46, X-31, X-73

TYPE OF TREATMENT	NO. OF PLANTS	FLOWERING RESPONSE
Short photoperiod plus short dark period*..	4	veg
Short photoperiod plus long dark period†..	18	mac fl & fr
Long photoperiod plus long dark period.....	10	mac fl & fr

* Other plants treated with similar results (*cf.* table 6).

† This treatment has always resulted in the subsequent development of flowers and fruits provided the night temperature is between 21° and 27° C. (*cf.* tables 9 and 10).

TABLE 8

EFFECT OF INTERRUPTION OF DARK PERIOD BY VARYING EXPOSURES TO LIGHT ON FLOWERING OF XANTHIUM. EXPERIMENTS X-43, X-60, X-71; UNTREATED CONTROLS ALL VEGETATIVE

LENGTH OF PHOTOPERIOD (HOURS)	LENGTH OF DARK PERIOD (HOURS)	LIGHT EXPOSURE DURING DARK PERIOD	NO. OF PLANTS	CONDITION OF PLANTS AT END OF TREATMENT
15.....	9	None	12	mac fl & fr
15.....	4.5+4.5	1 minute after 4.5 hours	10	veg
9.....	15	None	10	mac fl & fr
9.....	6.5+8	30 minutes after 6.5 hours	10	8 fl pr 2 veg
9.....	5+5+5	30 minutes every 5 hours	10	8 veg 2 infl pr
9.....	5+5+5	5 minutes every 5 hours	17	fl pr

the 9 hour dark period was divided into two 4.5 hour periods by exposure to artificial light (of 150 foot candles at the leaf surface) of one minute duration, the plants remained strictly vegetative.

In other experiments a 9 hour photoperiod was given and the 15 hour dark period was interrupted at 5 hour intervals, as shown in

table 8, by varying lengths of exposure to artificial light of 150 foot candles at the leaf surface. Control plants on 9 hour photoperiod and with an uninterrupted dark period of 15 hours all developed macroscopic flowers within 13 days. Of the plants which received one-half hour of artificial light at 5 hour intervals during the dark period, only two plants out of ten initiated flower buds. When only 5 minutes' exposure to light was given every 5 hours during the 15 hour dark period, all the plants initiated floral primordia. If the 15 hour dark period was interrupted by only one half-hour exposure to light (dividing the dark period into one 8 hour and one 6.5 hour periods), eight out of ten plants initiated floral primordia. Plants which received a 9 hour dark period remained strictly vegetative when a 1 minute period of exposure to light was given in the middle of the dark period. Thus at least thirty times as much light is required to negate the effect of a 15 hour dark period as is required to negate the effect of a 9 hour dark period.

Still another line of evidence supports the view that certain critical reactions involved in the initiation of floral primordia in *Xanthium* take place during the dark period. The temperature during the dark period greatly influences its effectiveness in relation to the initiation of flower buds. To test this, plants were removed from long photoperiod and given an 8 hour photoperiod at greenhouse temperature, which during the course of the experiments varied from 20° to 30° C. In the evening these plants were placed in the dark at temperatures ranging from 4° to 38° C. The following morning the plants were returned to the greenhouse, and a few of them from each lot returned to long photoperiod. This was repeated for ten successive dark periods. The results of one of three experiments are shown in table 9. All experiments gave essentially the same results. Plants given one short photoperiod at average greenhouse temperature, and given one long dark period at temperatures between 21° and 32° C., developed floral primordia after being returned to long photoperiod. Thus during one long night sufficient floral initiation substance may accumulate to result in the initiation of floral primordia. If the temperature during the dark period is maintained at 4° C., seven long dark periods are required to achieve the same result. Temperature

ranges between 4° and 21° C. could not be maintained accurately, but the experiment showed that the higher the temperature up to 21° C. the fewer the dark periods required. The temperature coefficient of the dark process cannot be determined precisely from this experiment, but in any case it is high, with a Q_{10} of approximately 3 and thus of an order which might well be expected of a biochemical process. Table 9 also shows that plants subjected to a temperature of 38° C. during the dark periods fail to initiate floral primordia even after four long dark periods. The optimum temperature for the most

TABLE 9

EFFECT OF VARYING TEMPERATURE DURING DARK PERIOD UPON FLORAL INITIATION BY XANTHIUM. EXPERIMENTS X-86 AND X-55. PLANTS AT GREENHOUSE TEMPERATURE DURING INTERVENING 9 HOUR PHOTOPERIODS. UNTREATED CONTROL PLANTS VEGETATIVE

Temperature (°C.)	Number of dark periods									
	1	2	3	4	5	6	7	8	9	10
4	veg	veg	veg	veg	veg	veg	veg	infl pr	infl pr	infl pr
21	infl pr	infl pr	infl pr	infl pr	infl pr	.....	.....	.....	.....	.....
27	infl pr	infl pr	infl pr	infl pr	infl pr	.....	.....	.....	.....	.....
32	9 veg, 2 infl pr	infl pr	infl pr	infl pr	infl pr	.....	.....	.....	.....	.....
38	veg	veg	veg	veg	.....	.....	.....	.....	.....	.....

rapid response to long dark period lies therefore between 21° and 32° C.

In contrast to the striking effect of varying temperature during the dark periods, varying temperature during the photoperiods exerts but little effect on the initiation of floral primordia, as is shown by the following experiment. Plants were subjected to a cycle of 8 hour photoperiods and 16 hour dark periods. The temperature during the dark periods was maintained at approximately 24° C. Some of the plants were exposed to a temperature of 4° C. during the photoperiod by placing them for 8 hours each day in a temperature-controlled room containing a carbon arc lamp which supplied an intensity of illumination of approximately 900 foot candles at the leaf surface. The remainder of the plants were subjected

to photoperiods at greenhouse temperatures of 20° to 30° C. Table 10 shows that floral primordia were initiated after exposure to one long dark period whether the preceding photoperiod was maintained at 4° or at 20°–30° C. Although low temperature during the photoperiod was thus without significant effect on the initiation of floral primordia, there was a marked decrease in the rate of subsequent development of the primordia.

There can be but little question that the flowering response of *Xanthium* to short photoperiod depends primarily upon reactions directly related to the dark period. It may be that the reactions

TABLE 10

Effect of low temperature during photoperiod on floral initiation by Xanthium. Plants at 24°C. during intervening 16 hours dark period (X-80). Untreated control plants vegetative. Dissection after 16 days

Temperature of photoperiod	No. of plants in each treatment	No. of short photoperiods before plants were again subjected to long photoperiod									
		1	2	3	4	5	6	7	8	9	10
4° C.	5	1 veg 4 infl pr	infl pr	infl pr	infl pr	infl pr	infl pr	infl pr	infl pr	infl pr	fl pr
Approximately 24° C. (greenhouse)	5	infl pr	fl pr	fl pr	fl pr	fl pr	fl pr	fl pr	mac fl & fr	mac fl & fr	mac fl & fr

leading to the manufacture of a floral initiating substance are adversely affected by low temperature. At least the responses of *Xanthium* indicate that, in any critical study of the relationship between temperature and photoperiodism, it is necessary to know the ranges of temperature during the dark period and during the photoperiod.

V. Nature of inhibition and localization

It has been shown in section III that if a portion of the *Xanthium* plant is subjected to short photoperiod, floral primordia are also initiated on other portions of the same plant although such portions

may be subjected during this same time to long photoperiod. This result might seem to disagree with the reports on localization of the flowering response in other plants (7). Undefoliated *Xanthium* plants, in fact, do not ordinarily exhibit the strict localization of response to short photoperiod which is found with undefoliated chrysanthemum plants. During the course of the present work a considerable number of experiments were carried out with chrysanthemum. The results were in harmony with those of others (2), namely, that if a portion of a leafy plant was subjected to short photoperiod, macroscopic flowers developed only on such portion,

TABLE 11

EFFECT OF VARIOUS TYPES OF DEFOLIATION ON INITIATION OF FLORAL PRIMORDIA BY RECEPTOR BRANCH. EXPERIMENTS X-24, X-37, X-82. 25 UNTREATED CONTROLS ALL VEGETATIVE

TYPE OF DEFOLIATION OF RECEPTOR BRANCH	UNDEFOLIATED	DEFOLIATED		
		COMPLETELY	EXCEPT FOR TWO MATURE LEAVES	EXCEPT FOR YOUNG EXPANDING LEAVES
Condition of receptor after 14 days	infl pr	infl pr	veg	fl pr
Total plants	25	24	24	26

while flowers completely failed to develop on the leafy portion subjected to long photoperiod. An attempt was made therefore to determine whether a localized response to short photoperiod might be obtained with *Xanthium*, and if so, under what conditions.

In these experiments, summarized in table 11, two-branched plants were used. The donor branches of all of them were not defoliated nor treated in any way except that they were maintained on short photoperiod. All the receptor branches were maintained on long photoperiod, but on different plants they were subjected to four types of treatment: (1) complete defoliation; (2) removal of the young expanding leaves at the tip, with continued removal of these as additional leaf primordia expanded; (3) removal of only the fully expanded leaves; or (4) not defoliated. Table 11 shows that only under one condition was a localized response to

short day obtained, namely, when the young expanding leaves had been removed from the receptor and only the fully expanded ones remained on it (fig. 8). Under these conditions floral primordia were initiated only on the donor whereas the receptor remained strictly vegetative. In all other cases, whether the receptor was completely defoliated or left leafy, floral primordia were initiated on both donor and receptor. If young leaves only were present on the receptor, floral primordia were initiated on it, and these developed into mature flowers and fruits more rapidly than did those on receptors which were either leafy or completely defoliated.

These effects resulting from the presence of immature and mature leaves on receptor branches which receive their stimulus for floral initiation from donor branches subjected to short photoperiod is in direct contrast to the condition prevailing when immature or mature leaves receive the photoperiodic stimulus by being directly subjected to a short photoperiod. In the latter case flower primordia are initiated only if mature leaves are exposed, and are not initiated if immature leaves only are present. The cause of this difference in behavior is not known. At present, largely as a matter of convenience, the failure to initiate and develop floral primordia on receptor branches where only mature leaves are present is referred to as an inhibitory effect of such leaves. The tendency to initiate floral primordia and the rapid development of these into flowers and fruits on receptor branches in the presence of young leaves is referred to as a promotive effect of such leaves.

This inhibitory effect of fully expanded leaves, and the promotive effect of young expanding leaves, on floral initiation on receptor branches were demonstrated repeatedly in separate experiments, as indicated in tables 11 and 12. The inhibitory effect of fully expanded leaves does not appear to depend upon the extent to which the length of the photoperiod exceeds the critical, but does depend upon the area of such leaves present upon any given branch or plant. This was shown in a series of experiments as follows: Donor branches of 70 two-branched plants were subjected to a 9 hour photoperiod. The receptor branches of 35 of these were subjected to a 16 hour photoperiod while the receptors of the remaining 35 were subjected to a 22

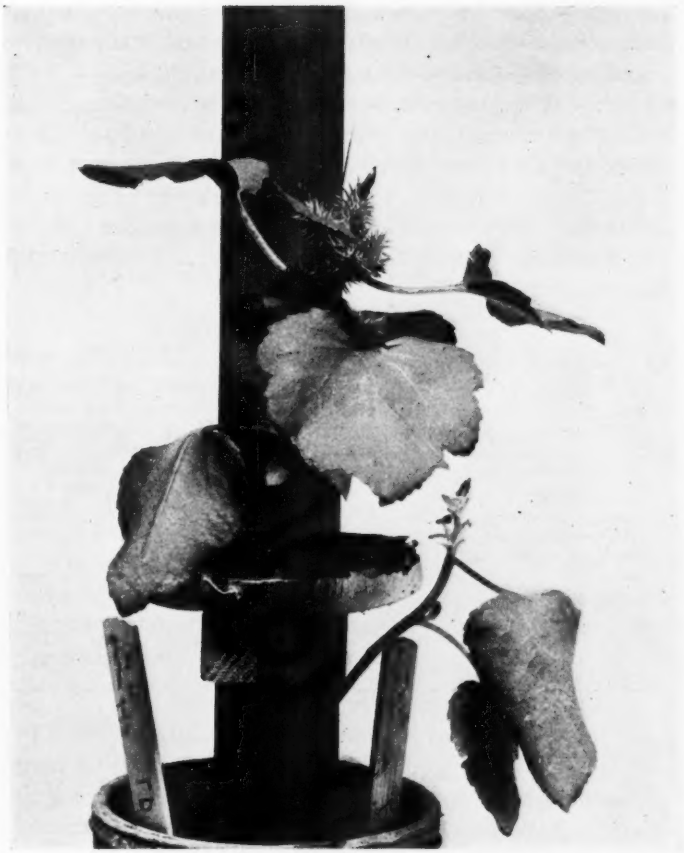

FIG. 8.—At the beginning of the experiment this plant was similar to the plant in fig. 7. The donor was treated in exactly the same manner, but the receptor had the young instead of the mature leaves removed, and was then continued on long photoperiod. The old leaves have prevented initiation of flower primordia and development of fruits on receptor but have exercised no measurable influence in development of flowers and fruits on donor.

hour photoperiod. Thus the first set of receptors received a photoperiod only one-half hour longer than the critical, while the latter set received a photoperiod 6.5 hours longer than the critical, and the dark period in the one was four times as long as in the other. Table 12 shows that if one-half or more of a fully expanded leaf is allowed to remain on the otherwise defoliated receptor, the receptor branch remains strictly vegetative both under a 16 hour and under a 22 hour photoperiod. One-fourth or less of a fully expanded leaf exerts no inhibitory effect upon the initiation of floral primordia under either condition.

TABLE 12

EFFECT OF VARYING LEAF AREA AND VARYING PHOTOPERIOD ON INHIBITION OF FLORAL INITIATION BY MATURE LEAVES. DONOR BRANCHES UNDEFOLIATED SUBJECTED TO 9 HOUR PHOTOPERIODS. PLANTS DISSECTED AFTER 14 DAYS; 10 UNTREATED CONTROL PLANTS ALL VEGETATIVE

PHOTOPERIOD TO WHICH RECEPTOR WAS EXPOSED (HOURS)	UNDEFOLIATED	NATURE OF LEAF ALLOWED TO REMAIN ON OTHERWISE DEFOLIATED RECEPTOR					COMPLETELY DEFOLIATED
		2 MATURE LEAVES	1 MATURE LEAF	½ MATURE LEAF	¼ MATURE LEAF	1 EXPANDING LEAF ¼″ LONG	
16	infl pr	veg	veg	veg	infl pr	fl pr	infl pr
22	infl pr	veg	veg	veg	infl pr	fl pr	infl pr

Since in the case of receptor branches the inhibitory effect of fully expanded leaves upon floral initiation is in opposition to the promotive effects of young expanding leaves, an experiment was performed in which only one leaf of an intermediate age was allowed to remain on the receptor. This leaf was initially approximately three-quarters of an inch in length and by the end of the 2 weeks' experimental period had developed into a fully expanded leaf. All younger leaves were removed as they began to expand. The single developing leaf, as may be seen in table 12, exerted the promotive effect characteristic of young leaves, and this effect does not depend exclusively upon the youngest leaves.

It has been shown that, under certain specific conditions, leaves on long photoperiod are capable of suppressing the initiation of floral

buds. The question arises as to whether or not this inhibitory effect is localized. In the experiments given in tables 2, 11, 12, 15, and 16, it was found that on two branched plants the number of floral primordia initiated and the rate of their subsequent development on the donor branch were entirely unaffected by any treatment given

TABLE 13

EFFECT OF APPROXIMATELY EQUAL AREAS OF YOUNG EXPANDING LEAVES OR OF FULLY EXPANDED LEAVES ON INITIATION OF FLORAL PRIMORDIA BY XANTHIUM PLANTS SUBJECTED TO SHORT PHOTOPERIOD (X-77). UNTREATED CONTROLS VEGETATIVE. DISSECTION AFTER 17 DAYS

PLANTS DEFOLIATED EXCEPT FOR	NO. OF SHORT PHOTOPERIODS						
	1	2	3	4	5	6	7
Portion of one mature leaf of about 2 sq. cm. area	veg	veg	fl pr	fl pr	fl pr	fl pr	fl pr
One young expanding leaf about 2 sq. cm. area	veg	veg	veg	veg	veg	veg	veg
Undefoliated control	infl pr	fl pr	fl pr	mac fl & fr	.....	.....	.....

the receptor branch. In these experiments the effect of the leaves on long photoperiod has been apparently confined to the branch exposed to long photoperiod.

VI. Relative response of various buds on a plant

On any given plant placed upon short photoperiod, a greater total number of flowers and fruits will develop rapidly if the terminal bud is removed. When the terminal bud of a vegetative plant is removed, many lateral buds start to expand at once, and these buds immediately develop flowers and fruits if the plant is on short photoperiod. A similar result is obtained if, instead of removing the terminal bud, the young expanding leaves are removed.

The several buds on any given donor or receptor do not respond alike. In general the buds on the donor branch respond more rapidly and more of them develop into mature fruits than do those of the receptor branch. On any plant, however, the terminal growing point of any branch responds more rapidly than the primordia of axillary buds. Thus if the terminal bud of the donor branch is removed at

the start of treatment, the terminal growing point of the receptor branch responds more rapidly than do the axillary buds of the donor.

Since the inhibition of the expansion of axillary buds seems to be associated with the presence of young expanding leaves near the tip of the axis, as described, and since the presence of young expanding leaves on the receptor of a two branched plant seems to produce some promotive effect upon the development of flowers and fruits by the receptor branch (section V), it is possible that young leaves, through their ability to supply auxin, produce both results. To test this possibility the following experiments were carried out: An attempt was made to replace by the use of indoleacetic acid the promotive effect of young expanding leaves on floral initiation. The receptor branches of two branched plants were defoliated either completely, or by removal of the young expanding leaves at the tip. Indoleacetic acid in lanolin paste (1 and 0.2 per cent) was then applied to the petiole stumps of the youngest three leaves. Indoleacetic acid applied in these high concentrations was completely without effect on either the initiation of floral primordia or on the development of flowers. Receptor branches, with mature leaves only, remained vegetative even if supplied with indoleacetic acid, as described, and flowers did not develop more rapidly on completely defoliated branches so treated than on untreated defoliated branches.

In another experiment similar high concentrations of indoleacetic acid as a lanolin paste were applied along the entire length of the stem, and the number of long dark periods necessary for the initiation of floral primordia was compared with the number necessary for untreated control plants. It was found that the untreated control plants, those treated with plain lanolin paste, and those treated with 0.2 per cent indoleacetic acid, all flowered on subsequent long photoperiod if they were subjected to one or more long dark periods. Plants treated with 1 per cent indoleacetic acid in lanolin, however, flowered only if given two or more long dark periods and not when given one dark period. No effect upon the relative sensitivity of the various buds was found. Thus, although auxin may play some role in the inhibition of flower bud initiation, still this effect is not

marked, when indoleacetic acid is applied. In numerous experiments carried out as described in section IX, indoleacetic acid was also without activity in initiating floral primordia in *Xanthium*. What relation this may bear to the well known effect of indoleacetic acid in suppression of bud development was not investigated.

VII. Nature of induction

In the preceding sections consideration has been given mainly to the initiation of floral primordia by *Xanthium*. It has been observed during these experiments, as well as in those of others (4), that initiation of floral primordia by the influence of suitable photoperiod is accompanied by other striking aftereffects when the plant is subsequently transferred to other photoperiods.

If a vegetative *Xanthium* plant is removed from long photoperiod, subjected to one long dark period under suitable environmental conditions (section IV), and returned to long photoperiod, this plant will initiate floral primordia and will develop macroscopic flowers after approximately 14 days. During the single long dark period no histologically detectable change occurs in the plant. Nevertheless some change has been brought about, a change which results in its flowering when grown subsequently on long photoperiod. This phenomenon, which is essentially one of photoperiodic aftereffect, may be referred to as photoperiodic induction. Induction in this sense is not the initiation of floral primordia per se, but is rather the change within the plant which takes place in relation to an alternation of photoperiods and dark periods which results in the initiation of floral primordia. Under suitable conditions of temperature this change may be brought about in *Xanthium* by subjection to one or more long dark periods. Subsequent flowering results whether the photoperiods following the long dark period are long or short. Induction appears to be associated with some alteration in metabolism, and experiments have been performed to investigate the nature of this alteration.

Plants which have undergone induction continue to supply the floral initiation substance when subsequently subjected to long photoperiods. This was demonstrated by the use of plants which

had been induced by exposure to 24 short photoperiods, and which were approach grafted to defoliated vegetative plants. Both plants were then allowed to remain on long photoperiod. The results are shown in table 14.

This ability of induced plants to continue to supply the floral initiation substance when such plants are returned to long photoperiod, might be attributable to (a) the storage of reserves of it during the exposure to short photoperiod and usage of this supply during the following long photoperiods, or (b) an alteration of the metabolism of the plant such that it manufactures the substance even

TABLE 14

PLANTS INDUCED BY EXPOSURE TO SHORT PHOTOPERIOD CONTINUE TO SUPPLY FLORAL INITIATION SUBSTANCE EVEN WHEN SUBSEQUENTLY SUBJECTED TO LONG PHOTOPERIOD. UNTREATED CONTROLS VEGETATIVE

EXPERIMENT NO.	TREATMENT	NO. OF GRAFTS	TIME OF OBSERVATIONS	CONDITION OF RECEPTOR
X-12 and X-17	Photoperiodically induced donor grafted to vegetative receptor	8	14–22 days	6 infl pr 2 veg

when subjected to long photoperiods. A final decision between these two alternatives is not possible at the present time, but a number of considerations indicate that the latter may be the more probable, as is illustrated by the following experiment: Small vegetative *Xanthium* plants were subjected to seven short photoperiods during and after which periods they initiated floral primordia and bloomed. They were then grown under continuous illumination for 7 months. After this they continued to receive long photoperiods for a total duration of more than one year. During this period the plants increased in size 100 or more times, and produced many new shoots. They also continued to produce flowers, although new axillary shoots occasionally developed vegetatively for a time. It would seem unlikely that floral initiation and flower development substances stored in the young plants during the seven consecutive short photoperiods could be responsible for this striking aftereffect. It seems more prob-

able that these plants continued to manufacture the substances in question during the exposure to long photoperiods. These plants have in effect become indeterminate or everblooming with respect to photoperiod.

It has been a common experience during the course of the present work to find that the development of floral primordia into mature flowers is much slower for a plant which has been induced by one long dark period, than for one given a succession of long dark periods. Thus the rapidity with which a plant supplies the sub-

TABLE 15

EFFECT OF VARYING EXPOSURES OF DONOR BRANCH TO SHORT PHOTOPERIOD ON DEVELOPMENT OF FLOWERS AND FRUITS ON RECEPTOR AFTER REMOVAL OF DONOR. DISSECTION AFTER 21 DAYS

EXPERIMENT NO.	NO. OF SHORT PHOTOPERIODS TO WHICH DONOR WAS EXPOSED	NO. OF PLANTS	SUBSEQUENT DEVELOPMENT OF FLOWERS AND FRUITS ON RECEPTOR
X-53	2	5	veg
X-53	4	5	fl pr
X-53	8	5	fl pr
X-25	11	4	fl pr
X-24	27	20	mac fl & fr

stances necessary for floral development seems to depend to a certain extent upon the length of the induction period.

The ability to supply these substances necessary for floral development seems to be transferable from one branch to another. This has been demonstrated in several experiments in which a number of lots of two branched plants were used. In these the donor branches of each lot were subjected to a definite number of short photoperiods. Then the donor branch was severed from the plant, while the receptor branch was in every case maintained under conditions of long photoperiod. The development of flowers and fruits on these receptor branches was then observed at intervals. The results of three such experiments are given in table 15. If the donor received but two short photoperiods before it was removed, the receptor remained strictly vegetative, even after 30 days. If, however, the

FIG. 9.—Receptor branch of two branched plant of which the donor received 27 short photoperiods and was then removed. At the time of removal, all macroscopic flowers and fruits were removed from the receptor (fruits shown have developed subsequent to that time). Photograph taken 40 days after removal of donor.

donor received four short photoperiods and was then removed, floral primordia were subsequently initiated on the receptor, and these primordia continued to develop somewhat further, although slowly. In one experiment the donor received twenty-seven short photoperiods before it was removed. During this period the receptor had developed macroscopic flower and fruits. All such flowers and fruits were removed from the receptor when the donor was cut off. Forty days later numerous flowers and fruits had again developed on these receptor branches, which had themselves never been subjected to short photoperiod. An example of such a receptor is given in figure 9. It is evident that if the donor branch is exposed to a sufficient number of short photoperiods the receptor may behave in some respects as if directly photoperiodically induced; that is, changed in a manner which results in the continued production of flowers and fruits even when it is subjected to uninterrupted long photoperiods.

Such indirect induction at least partially depends upon the leaves of the donor and is accelerated if the buds on the donor are removed (fig. 10*B*). The plants shown are of a series whose donors received eleven short photoperiods. At the end of this time all the receptor branches possessed floral primordia. The plants were then divided into four lots and the donor branches of any given lot were subjected to one of the following treatments: (1) severed from the receptor, (2) disbudded and exposed to long photoperiod, (3) defoliated and exposed to long photoperiod, and (4) left intact and exposed to long photoperiod. In figures 10 and 11 a typical receptor of each group is shown after a development of 40 days following the respective treatments. In the cases where the buds of the donor were removed but the leaves allowed to remain, the receptor possessed numerous large fruits. Where the donor had been removed, the buds of the receptor had developed but little. The other two cases exhibit intermediate development of flowers on the receptor. Removal of the buds on the donor at the end of the short photoperiod treatment thus greatly accelerates the subsequent development of flowers and fruits on the receptor.

In another experiment 40 two branched plants were used. These

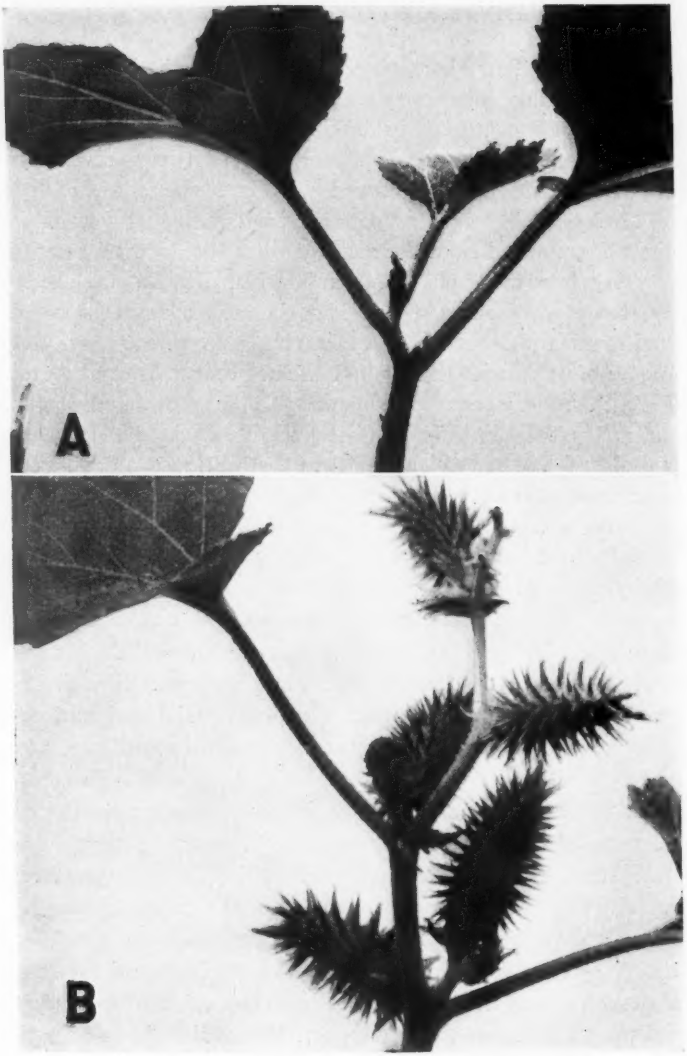

FIG. 10.—*A:* receptor branch of two branched plant whose donor received 11 short photoperiods and was then immediately removed. *B:* receptor branch of a two branched plant whose donor branch after having received 11 short photoperiods, was not removed but had its terminal and all its axillary buds removed. Note that although both receptors were grown continuously under long photoperiod, *A* has macroscopic flowers only and *B* well developed burs. Photograph taken 41 days subsequent to beginning of treatment.

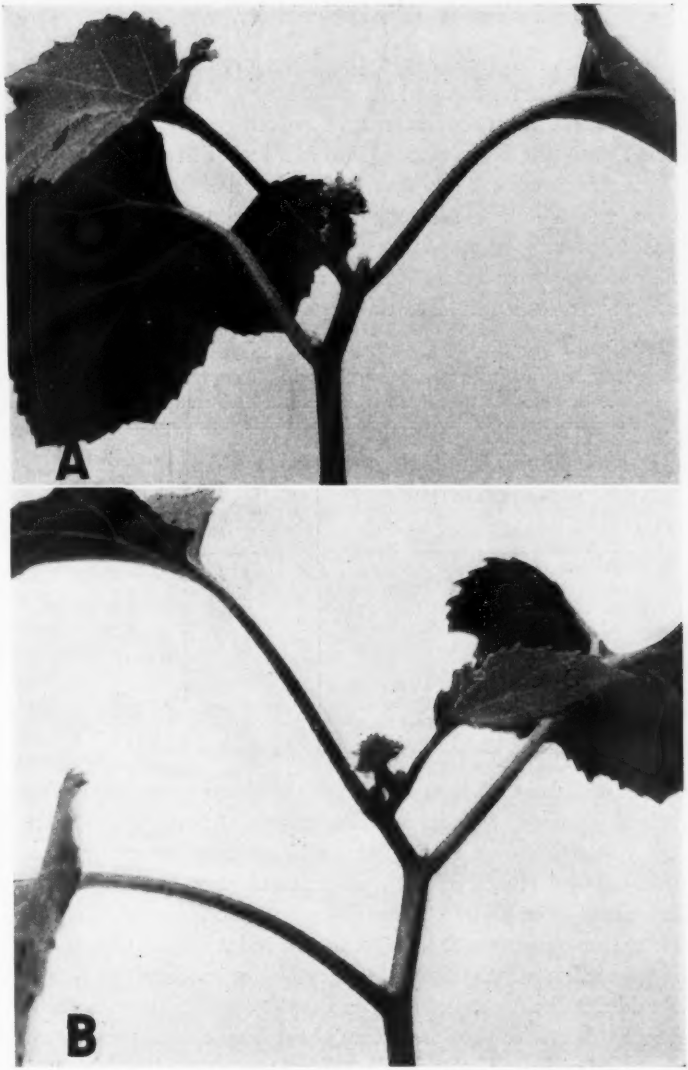

FIG. 11.—*A:* receptor branch of two branched plant whose donor received 11 short photoperiods and was then defoliated. *B:* receptor branch of two branched plant whose donor branch received 11 short photoperiods and was not subjected to further treatment other than being continued on long photoperiod. Note that *A* and *B* are intermediate with respect to floral development between *A* and *B* of fig. 10.

were divided into two groups; the donor branches of one group received two short photoperiods and those of the second group received four. After the treatment of a given group with short photoperiod, each group was divided into four lots of five plants each, and the donor branches of any given lot were subjected to one of the following treatments: (1) severed from the receptor, (2) disbudded and exposed to long photoperiod, (3) defoliated and exposed to long photoperiod, or (4) left intact and exposed to long photoperiod. The results are recorded in table 16. Regardless of the subsequent treatment of the donor, all the receptors of the plants on which the donor received four short days initiated floral buds. All receptors remained strictly vegetative on those plants whose donors were removed or defoliated and placed on long photoperiod after these donors had received only two short photoperiods. The receptors initiated floral buds in those cases where the donors received two short photoperiods and were subsequently exposed to long photoperiods, either intact or disbudded. The difference in floral development in these cases indicates that leaves of the donor which have been exposed to an induction period of short photoperiod continue to supply substances which bring about initiation and development of floral primordia in the receptor, even after these leaves are subsequently placed on long photoperiod.

TABLE 16

EFFECT OF VARIOUS TREATMENTS OF DONOR ON DEVELOPMENT OF FLOWERS AND FRUITS BY RECEPTOR. EXPERIMENT X-53

TREATMENT OF DONOR		NO. OF PLANTS	DEVELOPMENT OF FLOWERS AND FRUITS ON RECEPTOR AFTER 21 DAYS
Two short photoperiods then placed on long photoperiod and	Cut off	5	veg
	Defoliated	5	veg
	Disbudded	5	fl pr
	Left intact	5	fl pr
Four short photoperiods then placed on long photoperiod and	Cut off	5	fl pr
	Defoliated	5	fl pr
	Disbudded	5	mac fl & fr
	Left intact	5	fl pr

VIII. Attempts to determine nature of floral initiation substance

On the basis of the observation that the floral initiation substance *diffuses* from a plant subjected to short photoperiod into a vegetative plant exposed to long photoperiod and brings about the initiation of floral primordia by the latter, attempts were made to extract the floral initiation substance from leaves which had been subjected to short photoperiod, and by the use of such extract to bring about floral initiation in vegetative plants or cuttings.

Several test methods were employed for supplying such extracts to vegetative plants maintained on long photoperiods. A desirable

TABLE 17

RESPONSE OF XANTHIUM CUTTINGS TO LONG AND SHORT PHOTOPERIODS

EXPERIMENT NO.	TREATMENT	NO. OF CUTTINGS	CONDITION OF CUTTINGS AFTER 14 DAYS
X-14 and X-29	Short photoperiod	15	fl pr
	Long photoperiod	10	veg
	16 hour photoperiod	10*	veg

* Approximately 4500 cuttings have been subjected to 16 hour photoperiod and have remained strictly vegetative in other experiments.

method would be one in which vegetative shoots kept on days longer than the critical would be able to take up the solution to be tested. It is also necessary that the control plants remain vegetative. Cuttings were used most extensively as test material. Sturdy vegetative plants 3–4 weeks old were severed above the cotyledonary node, and defoliated below the uppermost partly expanded leaf. These cuttings were tied in groups of four or five and placed in 20 cc. shell vials containing the solutions to be tested. It is shown in table 17 that if such cuttings are placed in water and exposed to short photoperiod, they respond readily in the production of floral primordia and of macroscopic flowers. When similar cuttings were subjected to 16 hour photoperiod or to long photoperiod they remained strictly vegetative. Cuttings respond to short day with floral initiation just as do intact plants, and it would seem justifiable to make use of such

cuttings as test material for the detection of the floral initiation substance.

In all of the cutting tests to be described, the cuttings were maintained under either a 15.5 or a 16 hour photoperiod. The humidity was adjusted so that approximately 10 cc. of solution per four cuttings per day was lost by transpiration. That solutions or extracts supplied to the cuttings were actually taken up was shown by the marked toxic effects of certain solutions upon the upper leaves of the cuttings, and by the marked effects which certain solutions exerted on vegetative growth (stem elongation, rooting, etc.). In most cases the solutions were renewed daily.

The vials containing the cuttings were placed in numbered racks each holding ten or twenty vials. It was found possible to test as many as twenty-five racks (250 solutions) at one time. After a standard arbitrary time of two weeks under any particular test, the cuttings were dissected under the binocular microscope and inspected for floral primordia. As shown in table 16, this period is approximately twice as long as is required for the initiation of detectable floral primordia by similar cuttings subjected to short photoperiod.

A number of the substances known to possess activity as plant growth factors were investigated by the cutting test for possible activity in floral initiation. Thus vitamins B_1, B_2, B_6, ascorbic acid, pantothenic acid, nicotinic acid, inositol, indoleacetic acid, theelin, and theelol were used over wide ranges of concentrations. Yeast extract, which contains several of these growth factors, was also used. All these substances were found to be completely without activity in initiating floral primordia on *Xanthium* cuttings. Vitamin B_1, theelin, and yeast extract were also supplied in the nutrient solution given to *Xanthium* plants in sand culture and maintained under long photoperiod. Despite the great promotive effects of these materials on growth, the plants remained strictly vegetative. In another experiment vitamins B_1 and B_6, pantothenic acid, and nicotinic acid were supplied to vegetative *Xanthium* plants through funnels of appropriate size attached to the stump of the decapitated epicotyl. These plants also remained vegetative. It seems probable that the substance responsible for floral initiation is not identical with any of these known plant growth factors.

Vitamin B_1, ascorbic acid, theelin, theelol, and yeast extract were also investigated for possible promotive effects upon the development of chrysanthemum flowers. Plants of a late flowering clone were subjected to eight short photoperiods, after which they were found to have microscopically detectable floral primordia. They were then returned to long photoperiod, transplanted to sand, and supplied with nutrient solution containing various concentrations of the above growth factors. No effect upon the continued development of the microscopic floral primordia into macroscopic flowers was found in any case.

Since the work of GARNER and others (**8**) has shown that the initiation of the flowering state by appropriate photoperiod is associated with changes of acidity, a number of the common organic acids found in plants were tested, each over a wide range of concentrations, for possible effects on floral initiation on *Xanthium* cuttings. Succinic acid, malic acid, tartaric acid, citric acid, fumaric acid, oxalic acid, and maleic acid were found to be without effect, as were also the amino acids arginine, lysine, and glutamic acid.

Two hundred and forty-six different kinds of extracts and extract fractions were made during the course of attempts to extract the floral initiation substance from leaves. Extraction with several solvents, such as water, ethyl alcohol, ether, chloroform, acetone, benzene, and pyridine, was employed, and both fresh and ovendried leaves were used. Considerable precaution was exercised in the treatment of these extracts. All concentrations and evaporations were done in an atmosphere of N_2 under diminished pressure and below 60° C. in general, to decrease possible oxidation of the active material. Each extract was tested in several concentrations, so that more than 1150 solutions were tested, and approximately 4500 cuttings dissected. About half of the extracts were of leaves from *Xanthium* plants which had been subjected to short photoperiod; but extracts of other short day plants, as well as of indeterminate and long day plants, all in the flowering state, were prepared. None of these extracts showed any activity in bringing about the initiation of floral primordia on *Xanthium* cuttings when tested in the manner described.

There are indications, however, that if the vegetative cuttings are

treated by slight modifications of the procedure here outlined, they may respond with the initiation of floral primordia to the application of a simple water extract of leaves from induced *Xanthium* plants.

IX. Discussion

The response of *Xanthium* to photoperiod appears to differ in some respects from that of certain other photoperiodically sensitive plants which have been investigated. The strict localization of the response which has been reported for leafy plants of cosmos (7), chrysanthemum (2), and of other plants, is not found in leafy plants of *Xanthium*. In the species other than *Xanthium*, leaves of shoots subjected to conditions of long photoperiod prevent the initiation of floral primordia upon such shoots, even though other portions of the same plant are subjected to conditions of short photoperiod. Localization of this kind can be obtained with *Xanthium* provided only mature, fully expanded leaves are allowed to remain on a receptor shoot subjected to long photoperiod. Mature leaves of *Xanthium* thus behave in this respect in a manner similar to the leaves of some of the other plants so far investigated. Young expanding leaves of *Xanthium*, on the contrary, appear to exert a promotive effect on floral initiation and flower development in receptor branches.

Xanthium differs from soy bean (1), chrysanthemum, and cosmos (7) in that exposure of a *Xanthium* plant to a number of short photoperiods sufficient to result in the initiation of floral primordia also results in the development of these primordia into mature flowers and fruits, despite subsequent maintenance under conditions of long photoperiod. Plants of the other species under similar conditions initiate floral primordia but these frequently do not develop into mature flowers or fruits unless exposed to a still longer induction period.

Xanthium plants initiate floral primordia after exposure to one long dark period at 21° C., irrespective of whether this dark period is immediately preceded by a long or a short photoperiod at a relatively low or relatively high temperature. On the other hand, soy bean (1) must be subjected to at least two long dark periods separated by one photoperiod, for the initiation of floral primordia. Evidently the initiation of floral primordia by *Xanthium* is the result of processes

taking place during the dark period. Whether or not other species or varieties of plants could be induced to initiate floral primordia during a single dark period under certain conditions of temperature or humidity remains to be determined. It may be that in some plants floral initiation is conditioned by processes taking place during the photoperiod as well as during the dark period.

On the basis of data presented in the foregoing experiments, the behavior of most short day plants and probably also of long day plants and many others can most readily be attributed to the presence of a floral initiating substance whose genesis is probably in the fully expanded leaves when directly exposed to appropriate photoperiods or dark periods. Such substance may be transported throughout the plant.

X. Summary

1. A description is given of a simple method whereby one portion of a plant may be subjected to one photoperiod while another portion of the same plant is being subjected to another photoperiod.

2. Floral initiation in *Xanthium pennsylvanicum* results if plants are subjected to photoperiods shorter than 15 hours with accompanying dark periods of longer than 8 hours. If *Xanthium* plants are subjected continuously to photoperiods longer than 16 hours with accompanying dark periods shorter than 8 hours they remain strictly vegetative.

3. The initial effect of the photoperiodic stimulus is perceived by the leaves which are subjected to short photoperiod. However, this stimulus, resulting in floral initiation, may be transported from these leaves to other portions of the same plant which are maintained under conditions of long photoperiod and may also move across a diffusion contact from a plant subjected to short photoperiod to a plant subjected to long photoperiod. The stimulus to floral initiation may therefore be attributed to a substance or substances manufactured in leaves subjected to short photoperiod.

4. The response of *Xanthium* to photoperiod is primarily a response to length of dark period rather than to duration of photoperiod. Thus reactions resulting in the formation of floral initiation

substances may take place during the dark period. These reactions are adversely affected by light and by low temperature.

5. Fully expanded leaves on receptor branches subjected to long photoperiod may exert some influence inhibitory to floral initiation; under similar circumstances young expanding leaves exert a promotive effect on floral initiation and flower development.

6. In *Xanthium* the development of mature flowers and fruits from floral primordia is also promoted by a substance or substances formed in portions of the plant which are exposed to short photoperiod and which may move across a diffusion contact. Whether or not this substance or substances is identical with the floral initiation substance has not as yet been determined.

7. A portion of a plant maintained under long photoperiod may be influenced by a portion of the same plant subjected to short photoperiods in such a way that it may behave as though it has been photoperiodically induced by direct exposure to short photoperiod. Flowers and fruits continue to develop on such portions of a branch which has never itself been subjected to short photoperiods.

8. Evidence is presented that the floral initiation substance is not identical with any of the following known plant growth factors: vitamins B_1, B_2, and B_6, ascorbic acid, nicotinic acid, pantothenic acid, theelin, theelol, inositol, or indoleacetic acid.

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CURRENT LITERATURE

Vergleichende Morphologie der niederen Pflanzen. Erster Teil: Formbildung. By BRUNO SCHUSSNIG. Berlin: Gebrüder Borntraeger, 1938. Pp. viii+382. Figs. 470. Rm. 36 unbound; Rm. 38 bound.

This comparative morphology of the lower plants considers the algae and fungi as a whole, and takes them up topic by topic instead of group by group. The present volume covers the vegetative structures; the second part will cover the reproductive organs, alternation of generations, sexuality, heredity, and evolution.

The book is divided into four sections, and in each of them much more attention is given to the algae than to the fungi. The first section, the comparative morphology of the cell, discusses the cytoplasm, the nucleus, the locomotor apparatus, the plastids, the vacuolar system, and the cell membrane. In each chapter there is a comprehensive account of the structures concerned for both the algae and fungi. In many respects this is the most satisfactory portion of the book, possibly because the general subject is one well adapted to the comparative viewpoint from which the whole book is written. However, one wishes that there were more direct references to the bibliography, which will appear in the following volume.

The second section has two chapters devoted to cell division, and one to a consideration of the cell as a whole. The third section, covering the comparative organography of the lower plants, sets up a series of types of plant body and proposes an elaborate new nomenclature for the various types. In it the plant bodies are analyzed according to their fundamental organization and are placed in three major categories. The first of these, the Nematoblasts, includes all those in which the plant body is resolvable to a single filament. The second, the Siphonoblasts, includes all those with a siphonaceous type of plant body. The third group, the Syngamata, includes all in which the plant body consists of several or many filaments. It is the equivalent of OLTMANN's "fountain type" of thallus. In the opinion of the reviewer this is the least satisfactory section of the book. One objection is the introduction of a new and elaborate terminology; a second and more important objection is the question of whether, when taken as a whole, the thallus construction among algae and fungi is a matter of fundamental significance. In certain respects the author's rather artificial classification of thalli of algae and fungi is reminiscent of the old herbalists' segregation of vascular plants into herbs, shrubs, and trees.

The concluding section is devoted to comparative anatomy, and discusses the

cortical system, the storage system, the medullary system, and the mechanical system of the thallus.

The problem of appraising the value of this book is an exceedingly difficult one. The first section will certainly be of service to those seeking information concerning the cytology of primitive plants. So far as the remainder of the book is concerned, one can say that it can be read to advantage only by botanists well acquainted with the algae.—G. M. SMITH.

The genus Septobasidium. By JOHN N. COUCH. Chapel Hill, North Carolina: University of North Carolina Press, 1938. Pp. ix+480. Illustrated. $5.00.

The acrid controversy concerning the dual nature of lichens is now recalled only as one of the curiosities of botanical history. So thoroughly intrenched has the newer view become that the recent demonstration of an even more striking relationship between a group of fungi and certain scale insects has failed to attract the attention it deserves. There are far fewer species of *Septobasidium* than of lichens, to be sure, and their distribution is more restricted, but they are both more abundant and more widespread in tropical and temperate regions than has generally been realized, and the relationship with the insect hosts is much more highly specialized than that between a lichen fungus and its associated alga. This relationship, first cautiously suggested by VON HÖHNEL and LITSCHAUER in 1907, has been investigated by a limited number of students, among whom the author of the present volume has been outstanding. He has now embodied the results of these studies in a beautifully illustrated work which summarizes in completely adequate fashion all that has been learned up to the present time concerning these highly significant forms.

The account of the symbiotic relationship between the fungus and the insect is necessarily based upon detailed observation and experiment involving only a limited number of species, but the evidence is overwhelming that the conclusions drawn are applicable, in all essential particulars, to the entire group. It is shown that the fungus is absolutely dependent upon parasitized insects for its nutrition, while at the same time it furnishes shelter and protection for the unparasitized individuals, without which these are scarcely able to maintain themselves. Dissemination of the fungus from host to host is apparently by the passage of both parasitized and unparasitized insects, presumably by crawling where the branches are in contact, or otherwise perhaps on the feet of birds. Woody plants bearing *Septobasidium* may be severely injured. The injury, however, is primarily due to the scale insects. The fungus shelters the insects, protecting them even against the effects of strong sprays, and is thus the effective factor in bringing about the damage.

On the basis of the symbiotic relationship, COUCH suggests that the Septobasidiaceae be raised to the rank of an order, coordinate with the Auriculariales, Uredinales, and Ustilaginales among the Heterobasidiomycetes possessing transversely septate basidia. He retains all species in the single genus *Septo-*

basidium, justifiably believing that this name should be conserved against the earlier and technically valid name *Glenospora*.

Of the 163 species, 5 varieties, and 2 hybrids described, all but a very few are illustrated, for the most part both by reproductions of photographs showing the habit and by figures of microscopic details. The account of one, at least, of the hybrids is far more convincing than references to hybrid fungi are wont to be. Fifty-six species, including four to which old herbarium names are affixed, are described for the first time. Technically they are not validly published, since Latin diagnoses are not supplied. But even those who strongly favor the Latin requirement can scarcely fail to recognize species so carefully described and illustrated.

It is noteworthy that a larger number of known species occurs in southeastern United States than in any comparable area. There can be little doubt that this is because most mycologists are inclined to pass over species of *Septobasidium* as sterile lichens, which many of them strikingly resemble. With more discriminate collecting, particularly in the tropics, it may confidently be expected that the number of species will be greatly increased.

In keeping with its character, the book has been meticulously edited and is singularly free from typographical errors.—G. W. MARTIN.

Micropedology. By WALTER L. KUBIËNA. Ames, Iowa: Collegiate Press, 1938. Pp. xvi+243. Illustrated. $3.00.

The book is a summary of some of the methods used and the results obtained by the author and collaborators during the past several years in a direct microscopic study of the soil. Fully half of the volume is devoted to the techniques employed. The use and development of incident-light microscopes, micromanipulations, soil preparation methods, and microchemical and optical methods of analysis are discussed. While these methods are primarily designed to aid in the study of mineral components, there are several worthwhile developments which should find application in biology.

A further large section of the book is devoted to the study of soil fabrics, by which the author means the "arrangements of constituents in the soil and their role in relation to each other." These studies, of more interest to the geologist and pedologist, serve as a background for the discussion of soil biology. The observations reported clearly demonstrate the influence of the microclimatic conditions upon not only the ecology, but even the size and character of the different soil inhabitants. These microhabitats are in a sense independent units, except for interrelations which make each habitat part of the same biological system. Application of the direct microscopic method to the characteristics of the microhabitats, to decomposition of organic matter and humus formation, and to the ecology of the soil flora and fauna is discussed. The method developed does not yet enable the direct observation *in situ* of bacteria, but KUBIËNA feels that this ideal may be soon accomplished. At present it is necessary to supplement

studies with the Rossi-Cholodny and Winogradsky technique. A brief survey of some of the preliminary results emphasizes the great potentialities of the method for a finer understanding of the role of both the plant and the microorganism in the biology of the soil.—W. W. UMBREIT.

Die Chromatographische Adsorptionsmethode. By L. ZECHMEISTER and L. V. CHOLNOKY. Vienna: Universitat Pecs. Julius Springer, 1937. Pp. 231. Illustrated. Rm. 14.40.

The rapidly increasing usefulness of chromatographic adsorption technique in the separation and purification of compounds makes this volume very welcome, especially to biological chemists. The authors quote in their preface "Jeder wissenschaftliche Fortschritt ist ein Fortschritt der Methode." In this discussion of adsorption methods, other workers should find many observations which will aid them in related problems.

The book is divided into a general discussion of the adsorption method and a more specialized section in which its application to specific mixtures of naturally occurring substances is discussed in detail. In the first section the relationship between order of adsorption on a chromatogram and constitution of compounds is discussed, especially the case of conjugated systems. The carotenoid pigments and derivatives provide the most familiar cases.

The second section includes detailed discussions of specific experiments and many literature references. Some groups discussed are: chlorophyll, carotenoids, flavins, anthocyanes, certain colorless compounds, plant and animal poisons, alkaloids, enzymes, vitamins, and hormones. An extensive table of summarized adsorption data on various carotenoids is presented.

The volume closes with a number of excellent photographs, in black and white, of adsorption columns. The entire book is full of illustrations, diagrams, and discussions of experimental detail, which highly recommend it, especially to those starting work with plant pigments.—F. P. ZSCHEILE.

Chromosome Number Relationship in the Leguminosae. By HAROLD A. SENN. Bibliographia Genetica, Vol. XII. Pp. 175–336. 1938.

This contribution represents a preliminary survey of the chromosome number relationships in the Leguminosae, including some 233 chromosome number determinations made by the author on 106 species of 33 genera, together with a list of the known chromosome numbers in 436 species of 74 genera in the family. The following haploid numbers have been determined: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 30, 32, 40, 48, 65. The basic number for the Papilionatae SENN considers to be probably 8.

Certain taxonomic changes are corroborated on the basis of the cytological findings, and the need for other changes is indicated. A hypothetical phylogenetic chart of the family, based on chromosome number relationships, and to a limited extent on morphological relationships, is presented. A primitive

8-chromosome papilionate stock seems to have given rise along at least four distinct lines to the present day Papilionatae. The 7-chromosome Vicieae probably arose as hypoploids from the primitive 8-chromosome papilionate stock, and the 11-chromosome Phaseoleae may have arisen through hybridization of a tetraploid of the 7-chromosome Vicieae line with a diploid of the 8 line.

Polyploidy is found to be low in the Leguminosae, only 23 per cent being found to be polyploids or derived from polyploids. The relation of polyploidy to geographic distribution is reviewed and discussed, and doubt is cast on the validity of the view that perennial and woody conditions are primitive. In general, the numerical data on chromosome numbers of the Leguminosae support MUNTZING'S conclusions of 1936 that higher numbers are associated with the perennial condition and indicate that in the Leguminosae, at least, some of the woody perennial species may have originated from herbaceous annual species.—J. M. BEAL.

Trees of Northeastern United States; Native and Naturalized. By H. P. BROWN. Boston: Christopher Publishing House, 1938. Pp. 490. Illustrated. $3.00.

This book is the result of the revision and enlargement of Technical Publication 15 of the New York State College of Forestry, Syracuse, N.Y., which appeared in 1921 under the title of Trees of New York State; Native and Naturalized. The book, written by an experienced teacher of dendrology and wood anatomy, is for teachers, botanists, foresters, and others who may wish seriously to study trees. It is scientific but non-technical.

The principles upon which the taxonomic classification of plants is based and a brief but clearly stated review of the morphology of woody plants are included. Readily usable separate keys, based on leaf, fruit, and twig characters, precede plates with legends and tabulated descriptive information for each of the 152 species figured. The illustrations are excellently drawn and are unusually complete in showing leaves, flowers, fruit, and winter twigs. Each species is carefully described under the following headings: habit, leaves, flowers, fruit, winter characters, habitat, range, and uses. The many excellent qualities of the book warrant its continued use for at least another twenty years by all who may need a tree manual for the northeastern United States.—C. F. KORSTIAN.

The Structure of Economic Plants. By HERMAN E. HAYWARD. New York: Macmillan Co., 1938. Pp. x+674. Figs. 340. $4.90.

During the last number of years there has been an increasing emphasis on the study and recording of the developmental aspects of plant anatomy in addition to the purely descriptive and phylogenetic phases. The former is of particular importance to all who must deal with the general problem of plant production, physiologists and pathologists. Studies of function are rendered far more direct and effective through a knowledge of structure. To know the pattern and course of development of a given plant species or variety is of utmost importance

to botanists and plant husbandmen alike. Often such patterns may be shifted through wide limits, both quantitatively and qualitatively, dependent upon the ranges of environment imposed. In applied terms this means that the yield and quality of the crop produced are in large measure the tangible results of a specific agricultural practice. In recognition of this fact, the author has laid particular stress on those parts of any given species which are of greatest economic importance, but has adequately treated the details of the entire plant as well.

The book is organized in two parts, the first dealing with general principles of plant anatomy and the second with the structure of sixteen plants of economic importance.

While occupying only one-sixth of the space, the chapters covering cells and tissues, the anatomy of the root, shoot, flower, and fruits, not only furnish an excellent introduction to general plant anatomy, but also acquaint the reader with the nomenclature which is to be used.

The author has not refrained from discussing controversial points and presenting the opposing views of critical investigators with reference to the morphological interpretation of certain tissue systems, flower and fruit structures. Such presentation, however, at no time overshadows a precise recording of tissue origins and detailed description of plant organs. The book brings together in one place for the first time the type of material long needed by workers in the broad field of plant industry. It is, however, far from a simple compilation of scattered data. Much of it is based on original investigations by the author, his coworkers and students, so that the work represents in its own right a distinct contribution to anatomical knowledge. A very considerable proportion of the illustrations are original and were prepared under the author's direct supervision. Many others are used for the first time outside the original publications. This liberal use of new material is particularly refreshing.

Part II deals in detail with sixteen crop plants—corn, wheat, onion, hemp, beet, radish, alfalfa, pea, flax, cotton, celery, sweet potato, white potato, tomato, squash, and lettuce. All are treated at length in a clear, direct style. This selection was determined by "the economic importance of the plant, its suitability as a representative of the family to which it belongs, and the intricacy of its anatomical and morphological detail."

The author states that he contemplates a companion volume dealing with important fruit crops. If it is as admirably done as the present one it will be enthusiastically received by all who work with plants.—J. H. GOURLEY.

Useful Trees and Shrubs. By FLORENCE BELL ROBINSON. Champaign, Illinois: The Garrard Press, 1938. $4.50.

This new venture in publishing consists of almost 500 4×6″ cards, each treating one species of ornamental tree or shrub. Cards of different colors are employed for the five groups of 206 deciduous shrubs, 148 deciduous trees, 62

conifers, 39 broad leaved evergreens, and 36 vines included. The data are necessarily of a general nature, expressing average conditions in the northeastern states and Canada. Statements concerning native habitat, hardiness, form and size, color at different seasons, root system, habitat preferences, periods of foliage, flowers and fruit, cleanliness, response to pruning, aspect, and value are included. Space is provided for sketches of summer and winter condition. Local variations may be noted on the back of the card. Nomenclature follows Standardized Plant Names. This file should be useful to students, amateurs, and professionals in the field of landscape architecture, and to nurserymen.—C. E. OLMSTED.

The Nation's Forests. By WILLIAM ATHERTON DU PUY. New York: Macmillan Co., 1938. Pp. xii+264. Illustrated. $3.00.

This book is a popular and well written elementary account of the nation's forests, their early exploitation, the creation of the National Forests, and the principles evolved for their management. The latter includes chapters on cutting practices, planting, sustained yield, erosion control, fire prevention, recreation, wild life, range management, and wilderness areas. Chapters on wood products and state and private forestry conclude the book. It is copiously and beautifully illustrated. More than half the pages are full-page halftones, the pictures largely obtained from the files of the U.S. Forest Service, which throughout receives its full measure of praise.—C. E. OLMSTED.